Vol. 3, No. 1 January 1960

PAKISTAN JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

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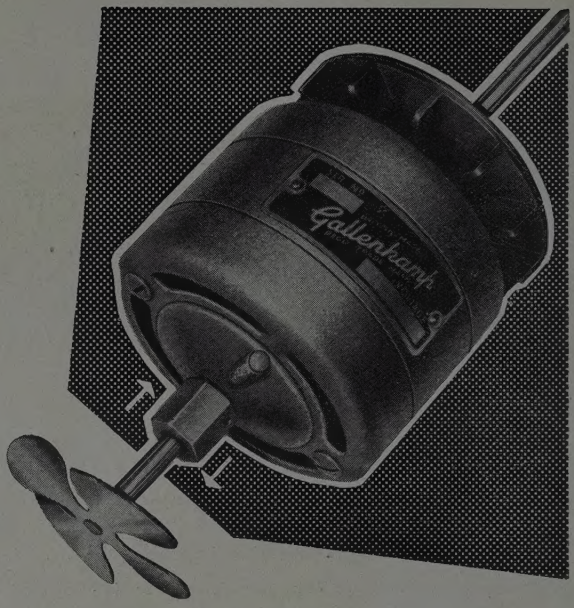

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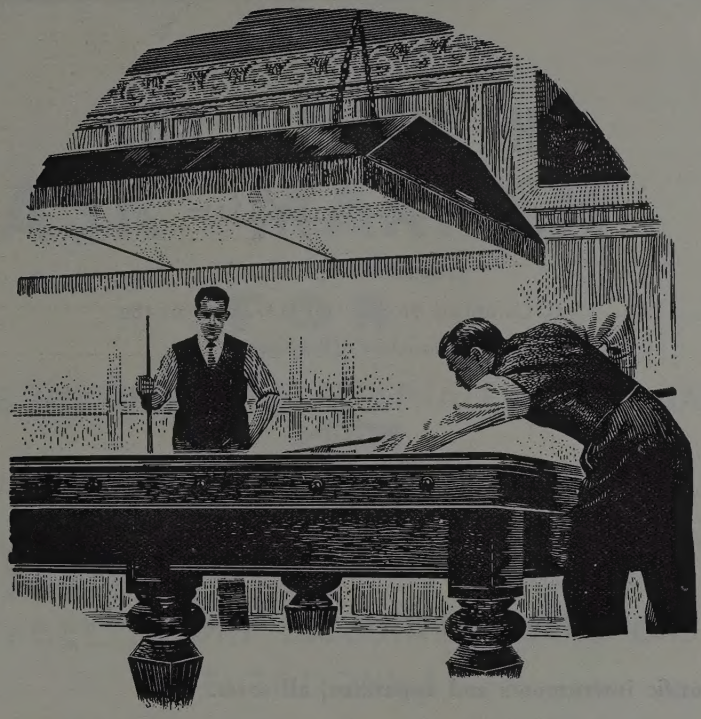

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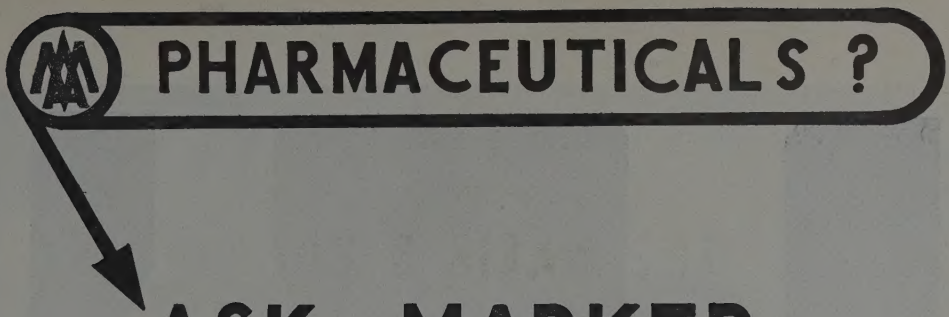

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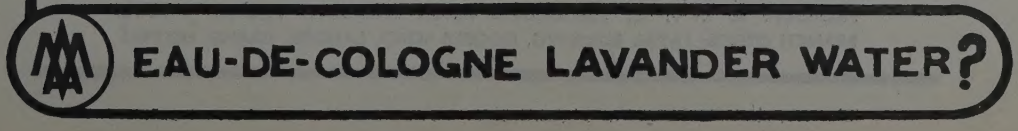

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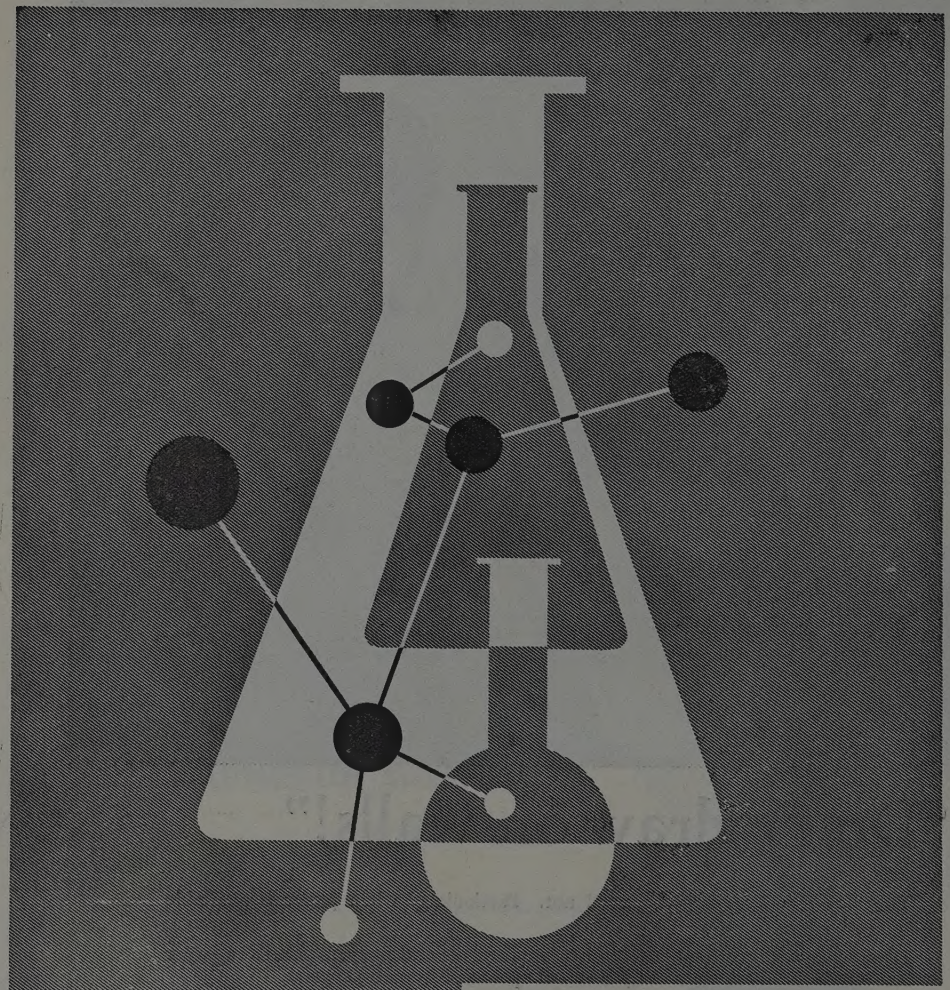

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Vol. 3, No. 1 January 1960

DETERMINATION OF NITROGEN IN ORGANIC COMPOUNDS WITHOUT DISTILLATION

Part I.—Determination of Ammoniacal Nitrogen in Organic Compounds

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(Received November 25, 1959)

The distillation step in the macro-determination of ammoniacal nitrogen in organic compounds by Kjeldahl method is eliminated. After having digested the sample in sulphuric acid with potassium sulphate and mercuric sulphate, the acid is neutralised. The resulting ammonium sulphate is treated with an excess of hypochlorite inpresence of potassium bromide. An excess of arsenite is then added and back-titrated with hypochlorite using bordeaux indicator.

Attempts have been made from time to time to modify the Kjeldahl method to make it applicable to all types of nitrogenous material and to improve its accuracy as well as to reduce the time of nitrogen determination. By eliminating distillation procedure from the Kjeldahl method, the process becomes easier and determinations can be made in shorter time. Elimination of the distillation step by titrimetric methods is based either on the oxidative treatment of ammonia or on the reaction of ammonium sulphate with formaldehyde[1] to yield stoichiometric amount of sulphuric acid. Physical methods mostly rely upon reactions resulting in colour formation of ammonia with various reagents. Nesslerisation is one such reaction.

Haanapal[2] has used Rupp and Rossler's method[3] to eliminate the distillation in the Kjeldahl method by oxidizing ammonia to nitrogen in an alkaline solution by means of a standard hypobromite solution. The excess of the latter is determined by adding potassium iodide and acid, and titrating the liberated iodine with thiosulphate. The procedure, however, has the serious disadvantage that hypobromite solution is unstable at room temperature and must, therefore, be kept below 5°C. Pappaport and Pistine[4] estimated nitrogen in blood, and Harvey[5] determined it in unicellular algae on the submicro scale using a hypobromite titration.

Belcher and Bhatty[6] applied Kolthoff and Strenger's[7] method involving titrations of ammonium salts with calcium hypochlorite, for determining nitrogen in organic compounds on micro and semimicro scales. The method is expeditious and is particularly suitable for multiple nitrogen determinations. Their method, however, cannot be used when the nitrogen in commercial commodities such as cereals, food-stuffs, drugs, etc., has to be determined. Also, facilities for a small-scale determination of nitrogen in most of the research and analytical laboratories are not always available. It was, therefore, considered desirable to modify the micro method of Belcher et al. (loc. cit.) to the macro for determining nitrogen without distillation.

The modified method has given satisfactory results for a number of organic compounds. The results obtained are given in Table 1.

The method was also used to determine nitrogen in the nitro compounds without using any reducing agent. The recovery of nitrogen from nitro compounds containing less than 10% of nitrogen was

TABLE 1.—DETERMINATION OF AMMONIACAL NITROGEN IN ORGANIC COMPOUNDS.

Compound	N Required	N found		
Ammonium sulphate	21.21	21.22	21.21	21.22
Hippuric acid	7.82	7.79	8.02	7.97
Acetanilide	10.36	10.22	10.43	10.36
Phthalimide	9.52	9.44	9.46	9.44
Phenacetin	7.82	7.87	7.91	8.02
8-Hydroxyquinoline	9.65	9.63	9.76	9.61
Benzamide	11.57	11.67	11.62	11. 4
Sym.-Diphenylthiourea	12.26	12.27	12.22	12.25
Uracil	24.98	24.93	24.89	25.01
Atropine	4.84	4.66	4.87	4.90
Brucine	6.50	6.47	6.43	6.46
Pilocarpine hydrochloride	11.45	11.27	11.23	11.24
Quinine sulphate ($2H_2O$)	7.16	7.22	7.25	7.18

almost quantitative. The recovery of nitrogen was not, however, quantitative when the amount of nitro-nitrogen was greater than 10%. The results for such determinations are recorded in Table 2.

TABLE 2.—DETERMINATION OF NITRO-NITROGEN IN ORGANIC COMPOUNDS WITHOUT PRE-REDUCTION.

	N required	N found		
o-Nitrobenzoic acid	8.38	8.12	8.11	8.12
5-Nitrosalicylic acid	7.65	7.59	7.52	7.58
p-Nitrobenzoic acid	8.38	8.18	8.20	8.11
2,4-Dinitrophenol	15.22	9. 0	9.08	9.05

A method, whereby greater percentages of reducible forms of nitrogen may also be determined without distillation, is being developed. Furthermore, since the macro-scale determination of nitrogen in organic compounds without distillation appears feasible, the method is being extended to cover nitrogen determination in commercial materials. Results of these findings will be communicated later.

Experimental

A. Reagents:

Sodium hypochlorite solution, 0.2 N
Arsenious oxide solution, 0.1 N
Bordeaux indicator (aqueous), 0.2%
Sodium hydroxide, 60 %
Sulphuric acid (d, 1.84), A.R.
Mercuric sulphate, A.R.
Potassium sulphate, A.R.
Sodium bicarbonate, A.R.
Potassium bromide, A.R.
Alundum (in pieces)

B. Procedure.—The compound (0.1 – 0.2 g.) was accurately weighed out into a 250 ml. Kjeldahl flask, and 5 g. of potassium sulphate and 0.6 g. of mercuric sulphate were added to bring about rapid digestion. The neck of the flask was washed down with 8 ml. of sulphuric acid. A few pieces of alundum were added to ensure smooth boiling. The digestion was carried out for 45 minutes if the nitrogen was present in the open chain and for 1½ hour if the nitrogen was present in a ring of the organic substance. After cooling, the digest was transferred to a 500-ml. conical flask with four washings. Sodium hydroxide solution was added drop by drop with occasional shaking until a yellow precipitate of mercuric oxide was formed. The solution was cooled and then totally neutralised by a gradual addition of sodium bicarbonate. Potassium bromide (4 g.) was then added and the flask shaken until the solution was clear.

An excess of sodium hypochlorite solution was added till the solution turned pale yellow. After 5 minutes, a known excess of arsenious oxide solution was run in and the excess was back-titrated with the hypochlorite solution using bordeaux indicator. The blank determination, using sucrose, was carried under similar conditions.

Calculation: 1 ml. 0.2 N NaOCl = 0.934 mg. N.

C. Discussion.—Normality of 1 N hypochlorite solution changed considerably at room temperature. It was found that 0.2 N solution of sodium hypochlorite in sodium hydroxide remained unchanged for about a week when kept in dark bottle at room temperature. For the sake of accuracy, however, hypochlorite was daily standardised before use.

A reversible indicator tartrazine was found suitable by Belcher[8] in the hypochlorite titration against sodium arsenite solution in low concentrations, but in the present investigations, where

concentrations of hypochlorite solution are higher, bordeaux was found more suitable indicator than tartrazine. On the present scale, when 0.2 N hypochlorite solution is used, the end point tends to be yellowish. As the colour of the titration solution with tartrazine is also yellow, the end point cannot be marked clearly. On the other hand, with bordeaux, the colour transition from pink to colourless is clearly discernible. When the compounds contain nitrogen in a chain such as in amines, amides, etc., the digestion proceeds with greater ease than when nitrogen is present as a part of a ring as in the case of uracil, 8-hydroxyquinoline, etc.

Nitro compounds which contain enough hydrogen in the molecule to form ammonia (containing less than 10% nitrogen) can be analysed by this method without pre-reduction.

As shown in Table 1, the method described yields good results in compounds having different nitrogen contents. The results compare favourably with those obtained by other workers in different ranges. The figures are within $\pm$ 0.2% of the expected results.

Acknowledgement

Our thanks are due to Dr. Karimullah, Director, West Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Lahore, for his helpful suggestions.

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A PRELIMINARY NOTE ON XANTHOXYLUM BUDRANGA FRUITS

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(Received April 27, 1959)

Xanthoxylum Budranga or bazna fruit has been examined. It yields an essential oil baznin from the calyx and a fixed oil from the fruit. The fixed oil has a consistency that is suitable as a raw material for soap, and consists of partly oleic acid and partly palmitic and stearic acids. Some amount of carbohydrate and protein are also available from the kernel powder.

Introduction

Xanthoxylum Budranga, locally known as Bazna, is a large thorny tree growing wild in certain parts of East Bengal, particularly in the districts of Mymensingh, Dacca, Sylhet and Tippera. It belongs to the family of *Rutaceae* and bears fruit in bunches during the rainy season. The fruits about 4 mm. in diameter are globular in shape and rather dark in colour when ripe. On drying, the upper skin of the fruit bursts into halves and the seeds tend to come out of the skin. These are reported[1] to contain about 17% of oil, which has been said to be consumed by the local people sometime as a substitute for mustard oil.

Through the courtesy of the Divisional Forest Officer, Mymensingh, we received some of these fruits last year, and these have now been examined. The fruits can be divided into two parts—calyx which covers the upper region of the dark coloured globular mass, consisting of the seed as an external cover. On examination, the calyx was found to give out a strong aroma, reminiscent of oil bergamot, indicating the presence of some essential oils. This was accordingly separated and the essential oil from it isolated, when a liquid product, consisting of about 1.2% of the weight of air-dried calyx was obtained. The oil has not yet been described by any one and we name it 'baznin'. It possesses a very characteristic pleasant odour, is optically active and shows a strong laevorotation. The quantity of baznin we have at the moment is small, but a more detailed study of its constitution is now underway.

Dry fruits after separation from the calyx were found to have an outer black shining coating under which a fluid material existed. On superficial examination when this skinny coating of the seed was broken, the fluid proved to be an oily substance, which was mistaken by earlier workers as watery fluid. On soaking the fruits in petroleum ether, the oil comes out into the solvent. Taking advantage of this dialytic process the seeds were steeped in petroleum ether at the ordinary temperature for some time, when parctically the whole of the fluid from within the seed coat was removed and was found to consist of about one-seventh of the weight of the seeds.

Next, the seeds were powdered and extracted with fresh quantities of petroleum ether when all the oil content of the kernel was extracted and this amounted to about one-sixth of the weight of the kernel.

After treatment of the kernel with petroleum ether, the residual powder was extracted with rectified spirit when about 2.5% of a semi-solid mixture was obtained.

The residue left was examined for protein and was found to contain some 11.08% of the same. It is expected that during the next season more of the fruit will be available to enable us to carry on the work towards completion.

Experimental

Isolation of the Essential Oil Baznin.—The dry calyx (15 g.) separated from the whole fruit was distilled in steam for about 2 hours. The distillate was saturated with common salt and extracted with ether. The extracts were dried with anhydrous sodium sulphate, filtered and distilled. Twenty such experiments were performed and a total of 36 g. of the essential oil could be collected, which represented 1.2% of the weight of the dry material employed. The oil thus obtained was fractionally distilled under reduced pressure. The first fraction boiling between 65-75°C. at 30 mm. or 40-41°C. at 8 mm. has $d_{28\cdot5}$, 0.8294; n_D^{29}, 1.4692; and $[\alpha]^{26}_D$, $-48.75°$.

Calculated for $C_{14}H_{22}O$: C, 81.55; H, 10.68. Found: C, 81.11; H, 11.00.

The second fraction was collected at 100-120°C. and 24 mm. pressure, and the third fraction was collected at 120-180°C. and 19 mm. pressure. These are now under a more detailed examination.

Fixed Oil in the Bazna Fruit.—(a) Whole dry fruits (500 g.) were kept immersed under petroleum ether (b.p. 40-60°C.) at the room temperature for 24 hours. The solution was then filtered and the solvent removed from it by distillation. Finally the oil was heated on a boiling water bath to remove the last traces of the solvent. By ten such extractions, a total of 647.5 g. of the oil was collected, which represented 12.95% of the lot of the fruit employed. This oil partially solidifies at the room temperature (33°C.) and has saponification value of 190.91 –191.3, iodine value of 60.2, R.M. value of 0.66 and contained 0.83% unsaponifiable matter. The oil consisted of the glycerides of several fatty acids described later.

(b) Next the kernel (500 g.) was finely powdered and extracted with fresh quantities of petroleum ether (40-60°C.) by cold percolation. The extracts were filtered, the solvent was distilled off and finally the residue was heated on a water bath to remove the last traces of the solvent. By ten such extractions, a total of 801 g. of oil was collected, which represented 16.02% of the material employed. This oil begins to solidify at about 20°C., and has saponification value of 181.91-183.4, iodine value of 113.9, R.M. value of 0.187 and contained 0.523% unsaponifiable matter.

Characterisation of the Fixed Oils.—(a) The oil from seed coat (31.3 g.) yielded 28.2 g. or 90.09% of mixed fatty acids on saponification with alcoholic caustic potash. These fatty acids have iodine value of 59.7 and neutralisation value of 209.3.

Fatty acid mixture (20 g.) was separated by lead salt method[2] into 6 g. or 30% of solid and 13.2 g. or 66% of a liquid acid mixture. The solid acids melted at 57.5-59.5°C., which appears to be a mixture of stearic and palmitic acids. The liquid acid has iodine value of 86.3.

(b) The oil from powdered kernel (30 g.) gave 25.6 g. or 85.3% yield of mixed fatty acids on saponification. These acids have iodine value of 115.3 and neutralisation value of 206.

These acids (20.3 g.) on separation as in other case gave 5.3 g. or 26.10% of a solid and 14.3 g. or 70.44% of a liquid acid mixture. The solid acid melted at 58-59.5°C. and has iodine value of 1.2, neutralisation value of 202.3, while the liquid acid has iodine value of 86.7 and neutralisation value of 177.5.

The solid acids on several crystallisations melted at 61-63°C. The molecular weight of these acids by silver salt method was found to be 271, which suggested it to be a mixture of 53% palmitic acid and 47% stearic acid. More detailed examination of the acids is in progress.

The residual pulp (1840 g.) on being extracted with rectified spirit gave 46.15 g. of a mixture of some crystalline solid and syrupy residue. This appears to be a mixture of carbohydrates and its detailed examination is in progress.

The rest of the pulp consisted of some protein (11.08%) along with some other products.

Acknowledgement

Our thanks are due to Dr. S. Siddiqui for his keen interest in the progress of the work, and to Mr. Safurul Haq and Mr. Manzurul Hossain for their help in analytical experiments.

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STUDIES ON TROMBIDIUM TINCTORUM LINN.

Part .I—Chemical Constitution of the Fat of Trombidium Tinctorum Linn.

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(Received October 1, 1959)

Various physico-chemical characteristics of the fat of *Trombidium tinctorum* Linn. have been studied. Relative proportions of solid, liquid and steam-volatile acids have been determined. The water-soluble and the water-insoluble steam-volatile acids were studied as such. The various solid and liquid fatty acids, separated by Twitchell's lead salt alcohol method, were determined after fractionation of their methyl esters under reduced presssure.

Introduction

The species *Trombidium tinctorum* Linn. (local name, bir bohiti) belongs to the members of the family *Trombidiidae* and is known as scarlet mites to the horticulturists. Its phylum is *Arthropoda*, order *Acarida* and class *Arachnida*.The members of this class are in fact spiders. It has been wrongly classified as cochineal insects by several authors.[1,2] The mites were identified as *Trombidium tinctorum* Linn. by Akhtar[3] in Pakistan and Evans[4] in England.

Interest in the investigations on *Trombidium tinctorum* Linn. has been evoked by the fact that the mites in the dried form are administered in the indigenous system for respiratory and reproductive ailments. The mites are reputed to possess nerve stimulating properties in Auyervedic and Unani schools of medicine. Their colouring matter is reported to possess sedative and anti-spasmodic properties. It is also useful in whooping cough, neuralgia, etc.

These mites become plentifully available during the rainy season, when they were hand-picked and preserved in rectified spirit for investigation.

Experimental

Extraction of the Fat.—The mites were extracted exhaustively with petroleum ether (b.p. 50-70°C.) in a Soxhlet apparatus. When most of the colouring matter had been removed, the mites were taken out from the Soxhlet apparatus, crushed in a mortar, and extracted further with petroleum ether (50-70°C.). The solvent was distilled off on a water bath. The residual fat thus obtained was intensely red and had a characteristic odour. It was dried first over anhydrous sodium sulphate and then at a temperature of 75-85°C.

Physical Characteristics.—The determination of various physical constants was carried out by following the standard procedures.[5] These values were: refractive index, 1.4575 (at 32°C.); specific gravity, 0.8923; and tint, equivalent to 9 units of yellow, 11 units of blue and 7 units of red coloured discs of the Lovibond tintometer.

Chemical Values.—The followng chemical values for the oil were determined by the usual methods[6]:—

Saponification value	=	202.50
Iodine value	=	80.10
Thiocyanogen value	=	58.27
Hehner value	=	94.40
Acid value	=	1.59
R.M. value	=	2.585
Polenske value	=	0.65
Kirschner value	=	2.87
Saturated acids (Bertram)	=	42%
Hexabromide value	=	0
Non-saponifiable matter	=	2.6%

Resolution of the Fat into Various Acid Fractions.—Eighty six grams of the fat was saponified with approximately 0.5 N alcoholic caustic potash solution. The alcohol was distilled and the soap dissolved in water. The non-saponifiable matter was extracted with diethyl ether in a continous liquid-liquid extractor. Afterwards the dried soap was thoroughly mixed with acid-washed white sand of 20 mesh and extracted with diethyl ether. This treatment removed almost all the colouring matter in the fat. The soap solution was then decomposed into fatty acids with dilute sulphuric acid. The liberated acids were steam-distilled to recover the steam volatile acids. The steam distillate was treated with ether and the ether-soluble fatty acids were recovered after distilling away the ether. The total amount of steam-volatile acids was determined from the titration of (1) the ether extraction residue, and (2) the ether treated aqueous distillate. The steam non-volatile acids were extracted with ether and dried in 'vacuum' at 100°C. They were separated into 'solid' and 'liquid' acids by Twitchell's lead salt alcohol method as adapted

by Hilditch.[7] Amounts and values of solid, liquid and steam-volatile acids are recorded in Table 1.

TABLE 1.—AMOUNTS AND VALUES OF STEAM NON-VOLATILE AND VOLATILE FATTY ACIDS

	Oil	Total acids	Liquid acids	Solid acids	Volatile acids
Amount	—	100	54.18	42.00	3.82
Saponification value	202.50	200.50	198.50	205.00	592.27
Iodine value	80.10	96.32	108.35	0.60	—

The composition of steam volatile acids was as follows:—

Acids extracted with ether from the steam distillate	=	2.48 g.
Saponification equivalent of the acids	=	94.72
Calculated amount of butyric acid (C_4)	=	1.29 g.
Calculated amount of iso-valeric acid (C_5)	=	1.18 g.

Liquid Acids.—The liquid acids were converted into their methyl esters which were fractionally distilled under reduced pressure and at elevated temperature, as recorded in Table 2.

The amount of individual esters and acids in the liquid acid fractions, as indicated in Table 3, was as follows:—

C_{14}	methyl ester	=	3.80 g.
C_{16}	methyl ester	=	20.48 g.
C_{18}	methyl ester	=	8.15 g.
C_{20-22}	methyl ester	=	0.18 g.
	Total	=	32.61 g.
or C_{14}	acid	=	3.67 g.
C_{16}	acid	=	19.40 g.
C_{18}	acid	=	7.76 g.
C_{20-22}	acid	=	0.17 g.
	Total	=	31.00 g.

Solid Acids.—Esterification of the solid acids to methyl esters was accomplished as recommended by Hilditch.[8] Eight successive fractions were collected by distilling the esters under diminished pressure and at elevated temperature, recorded in Table 4.

TABLE 2.—FRACTIONATION OF METHYL ESTERS OF LIQUID ACIDS. WEIGHT OF THE ESTERS DISTILLED = 32.63 g.

Fraction	Pressure	Temp.	Amount
L_1	1mm.	45- 65°C.	11.45 g.
L_2	,, ,,	65- 80°C.	8.12 g.
L_3	,, ,,	80-105°C.	7.00 g.
L_4	,, ,,	105-120°C.	0.82 g.
L_5 (residue)	,, ,,	120°C. falling	3.44 g.
		Total =	30.83 g.
		Loss =	1.80 g.

The loss in distillation was proportionately added to each fraction.

TABLE 4.—FRACTIONATION OF METHYL ESTERS OF SOLID ACIDS. WEIGHT OF ESTERS DISTILLED = 29.92 g.

Fraction	Pressure	Temp.	Amount
S_1	1 mm.	47- 58°C.	2.11 g.
S_2	,, ,,	58- 78°C.	1.04 g.
S_3	,, ,,	78- 88°C.	1.48 g.
S_4	,, ,,	88- 98°C.	4.10 g.
S_5	,, ,,	98-112°C.	4.78 g.
S_6	,, ,,	112-122°C.	6.56 g.
S_7	,, ,,	122-135°C.	4.67 g.
S_8 (residue)	,, ,,	135°C.-falling	4.95 g.
		Total =	29.69 g.
		Loss =	0.23 g.

TABLE 3.—VALUES AND COMPOSITION OF INDIVIDUAL ESTER FRACTIONS OF THE LIQUID ACIDS.

Fraction	Corrected wt. in g.	S.E.	I.V.	Composition			
				C_{14}	C_{16}	C_{18}	C_{20-22}
L_1	12.11	259.22	48.44	3.80	8.31	—	—
L_2	8.59	271.57	54.31	—	7.49	1.09	—
L_3	7.40	279.42	79.73	—	4.38	3.02	—
L_4	0.87	286.16	86.92	—	0.30	0.57	—
L_5 (residue)	3.65	297.38	91.87	—	—	3.47	0.18

The loss in distillation was added proportionately to each fraction. Saponification equivalents and iodine values for each fraction were determined from which mean molecular weight for each fraction was calculated, as recorded in Table 5.

The amount of individual esters and acids in the solid acids fractions was as follows:—

Methyl caproate	=	0.51 g.
Methyl laurate	=	2.979 g.
Methyl myristate	=	2.816 g.
Methyl palmitate	=	17.249 g.
Methyl stearate	=	6.370 g.
Total	=	29.924 g.
or Caproic acid	=	0.46 g.
Lauric acid	=	2.78 g.
Myristic acid	=	2.65 g.
Palmitic acid	=	16.35 g.
Stearic acid	=	6.07 g.
Total	=	28.31 g.

From the data recorded for liquid and solid acids, the composition of the fat can be represented as below:—

Glycerides of		
Butyric acid	=	2.48 %
Iso-valeric acid	=	1.34 %
Caproic acid	=	0.68 %
Lauric acid	=	4.12 %
Myristic acid	=	3.93 %
Palmitic acid	=	24.25 %
Stearic acid	=	9.05 %
C_{14} unsaturated acids	=	6.09 %
C_{16} unsaturated acids	=	32.33 %
C_{18} unsaturated acids	=	13.07 %
C_{20-22} unsaturated acids	=	2.80 %
Total	=	100.00

The component solid acids were calculated from an equation given by Hilditch.[9] The saponification equivalents of all the fractions were determined by the method of Marcali and Reimann III on the decigram scale.[10]

The determination of the individual unsaturated acids, constituting acids of C_{14}, C_{16}, C_{18}, C_{20} and C_{22} carbon contents, is difficult and misleading when based on iodine and thiocyanogen values or on bromo derivatives. This is so because while determining these values of one fraction it is not certain as to whether lower or higher unsaturated acids are present alongwith.

The derivation of the amounts of unsaturated acids is therefore entirely based upon the saponification equivalents of the ester fractions of the liquid acids.

In working out the composition of the fat, the non-saponifiable matter was not taken into consideration as the studies on this part are still in progress and will be followed up in Part II.

Acknowledgements

Helpful suggestions by Mr. Iftikhar Ahmad of West Regional Laboratories are acknowledged with thanks. The authors also wish to thank Dr. Salimuzzaman Siddiqui, Director of Scientific and Industrial Research, Karachi, for the keen interest he has shown in this problem.

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TABLE 5.—VALUES AND COMPOSITION OF INDIVIDUAL ESTER FRACTIONS OF SOLID ACIDS.

Fraction	Corrected wt. in g.	S.E.	I.V.	Composition				
				C_{10}	C_{12}	C_{14}	C_{16}	C_{18}
S_1	2.12	208.00	0.00	0.46	1.66	—	—	—
S_2	1.05	212.60	0.00	0.05	1.00	—	—	—
S_3	1.49	235.66	0.00	—	0.319	1.171	—	—
S_4	4.13	259.22	0.00	—	—	1.590	2.540	—
S_5	4.82	269.321	0.10	—	—	0.055	4.765	—
S_6	6.61	277.74	0.20	—	—	—	4.780	1.83
S_7	4.70	280.54	0.20	—	—	—	2.930	1.770
S_8 (residue)	5.00	285.50	0.50	—	—	—	2.230	2.770
Total:	29.92			0.51	2.979	2.816	17.245	6.37

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BIOCHEMICAL AND NUTRITIONAL STUDIES ON EAST PAKISTAN FISH

Part II.—Assessment of Dehydrogenase Activity in Fish Tissue and Investigation on the Mechanism of Fish Spoilage by this New Method

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A new method for the study of spoilage of fish has been developed. The dehydrogenase activity of the tissue has been investigated by noting the time of discharge of methylene blue according to Thunberg technique after allowing the tissue enzyme to act on the tissue substrates. On the basis of this new technique, 1 g. flesh of Koi, Singi, Bele and Hilsa showed slight activity whereas younger samples of Rohu, Katla, Kali Baus, Mrigale and Air and smaller species, Chapila and Puti showed almost negligible activity of dehydrogenase at the initial stage just after purchase from the market. On storage for 24 hours, a high rate of dehydrogenase activity was noted in each of the above fish samples—comparatively Singi, Koi, and Boyal showed less generation of activity than the others. The significance of these data and their relationship with the titratable acidity values as were presented in Part I of this paper have been discussed.

Introduction

In the previous paper by M. Q. Khuda, H.N. De and J. C. Debnath,[1] it was postulated that difference in the accumulation of acids in various species of fish at the initial stage and during spoilage, after storage, may be due to imbalance between activities of glycolytic enzyme system for pyruvic acid and lactic acid production and that of Kreb's cycle enzymatic chain of reaction for their complete utilisation.

In order to get clear information on this important aspect, a series of investigations have now been undertaken.

Although some work [2–5] has been done in the past on various constituents for determining fish spoilage, yet no attempt has yet been recorded on the above line so as to assess the mechanism of spoilage in a precise manner. The study of the above aspects of glycolysis and Kreb's cycle oxidation processes, which universally occur in every organism, involves the investigation of a number of dehydrogenases and oxidases for each of which a specially prepared substrate is required for laboratory experimentation. Nevertheless, it was thought worthwhile to study the laboratory enzymatic reaction under conditions almost identical with those present in the tissue cell structure by allowing the tissue enzyme extract to act on the natural substrates of the cell constituents. In the present case the activity of dehydrogenase has been studied in the above way. The basis for this technique was noted in our previous study on succinic acid dehydrogenase in spoiled fish tissue extract, in which it was observed that the blank experiment without any addition of the substrate, i.e., sodium succinate, elaborated almost equal activity as that conducted with the substrate. Details of the technique are given below.

Experimental

Thunberg Methylene Blue Technique [6, 7, 8].—According to this technique the oxidation of any substrate due to dehydrogenase system is measured by noting the time of decolorisation of methylene blue (M.B.) due to its reduction to leuco M.B. by hydrogen transfer from the metabolite under vacuum. In the present case a series of trial experiments were first conducted to ascertain the minimum amount of tissue, buffer mixture and methylene blue to be used for proper elaboration of enzyme activity. The following procedure was adopted.

In a series of Thunberg tubes, 0.9 ml. of a mixture of 8 ml. methylene blue solution and 6 ml. phosphate buffer (Sorensen pH 7.2) was introduced. Then 1 g. fish tissue, finely ground with a little quartz sand in 10 ml. buffer solution, was added to the mixture. Under ordinary condition the tissue extract is generally used for noting the enzyme activity, but in the present case the ground tissue along with the extract was also introduced into the tube so as to allow the enzymes to act on the substrates already present in the tissue. Thus the identical conditions as are present in the tissues for enzyme action have been maintained to a certain degree for the performance of "autodehydrogenation" process. The tubes were then evacuated for 1 minute by vacuum pump and incubated in a constant temperature bath at 37°C. The time of discharge of the methylene blue colour was then noted in each individual tube. Since the time of decolorisation is inversely proportional to the enzyme concentration, i.e., the activity, the reciprocal value of this time of discharge in minutes may be arbitrarily accepted as the relative autodehydrogenase activity per g. wet fish. But, since the moisture contents of eleven species of fish studied in the present investigation showed a wide variation from 64.66 to 82.57%,

the expression of the activity per g. wet basis as above and comparison and interpretation of these values will always lead to erratic conclusion. In order to make better judgement of the activities, the time of discharge per g. wet fish (T_1) has been corrected for expression on per g. dry basis (T_2) according to the following formula taking into consideration the moisture percentage of individual fish.

$$T_2 = T_1 \times \left(\frac{1 - \% \text{ moisture}}{100} \right)$$

From these values of T_2, i.e., time of discharge, for 1 g. dry basis, the activity was then expressed as $1/T_2$.

Further, the initial values of titratable acidity and the fall in these values after 24 hours storage, as were reported in the previous paper, have also been included in the Table 1 (columns E and F) so as to correlate the dehydrogenase activity with these values.

As in the previous investigation, the fish was purchased from the market in the early morning and one sample of each species was sliced immediately in the laboratory and subjected to the above investigation. Another sample was stored for 24 hours in open (temperature, 84° to 90°F.) and the activities of these stored samples were noted next day.

Results

Table 1 shows that the fresh tissues of Puti, Chapila, Katla Naola, Rohu Naola, Kali Baus Naola, Mrigale Naola and Air did not discharge methylene blue colour even after incubation for a period of over 10 hours. This indicates that these fishes at the initial stage cannot elaborate a significant amount of dehydrogenase activity. Singi, Koi, Bele and Hilsa seem to possess some amount of dehydrogenase activity at the initial stage.

After 24 hours storage at ordinary temperature (84°—90°F.) in the laboratory, the methylene blue colour was discharged within a few minutes in all cases. Calculated on the basis of expected activity per g. dry basis, the time of discharge of methylene blue was 8.64, 10.44 and 10.15 minutes for Boyal, Koi and Singi, respectively, and from 2.44 to 4.07 minutes for the rest of the fishes.

TABLE I.—THE TIME OF DISCHARGE OF METHYLENE BLUE COLOUR (M.B.) OF FRESH AND STORED FISH AND THE RELATIVE DEHYDROGENASE ACTIVITY.

Local name of fish	Zoological name of fish	Moisture %	Fresh Fish	After 24 hr. - storage			Titratable acidity values (0.01 N) in ml. for fresh fish per g. dry basis	Fall in titratable acidity (0.01 N) in ml. after 24 hrs. per g. dry basis	D × F
			Discharge of M.B. (in hrs.) per g. wet fish	Discharge of M.B. (in min.) per g. wet fish	Discharge of M.B. (in min.) per g. dry basis	Enzyme activity per g. dry basis			
			A	B	C	D	E	F	
Singi	Palemon peneus	81.2	5 to 6 hrs.	54	10.15	0.098	27.37	6.29	0.61
Koi	Anabestestudineus.	76.26	,,	44	10.44	0.096	21.66	7.88	0.75
Baila	Glossogobius giuris	78.38	,,	40	8.64	0.115	27.19	10.64	1.23
Hilsa	Hilsa ilisa	64.66	,,	11	3.88	0.257	23.42	18.61	4.79
Puti	Barbus Puntius	79.14	18 to 24 hrs.	13	2.71	0.369	37.99	30.72	11.33
Rohu	Labeo rohita	82.57	,,	14	2.44	0.409	35.05	28.47	10.02
Air	Mystus Aor	80.2	,,	15	2.97	0.336	34.29	24.59	8.28
Chapila	Danoacquipinnatus	78.8	,,	15	3.18	0.314	40.00	34.15	10.73
Kali Baus	Labeo nandina	74.54	,,	16	4.07	0.245	31.34	18.62	4.57
Mrigale	Cirrhina Mrigala	81.11	,,	17	3.21	0.311	48.35	33.64	10.48
Katla	Catla catla	78.86	,,	19	4.01	0.248	33.55	23.36	5.82

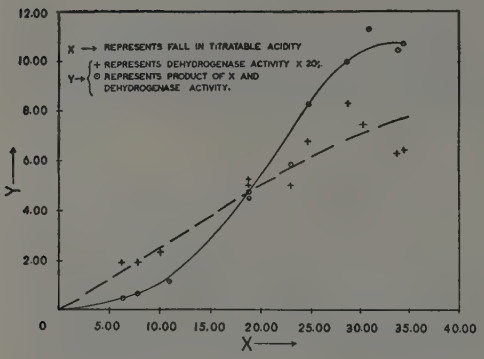

Fig. 1.—The relationship between dehydrogenase activity and fall in titratable acidity.

Figure 1 shows graph for (i) dehydrogenase activity (crosses) against the fall in titratable acidity, and (ii) the product D×F (in Table 1) against this fall in titratable acidity.

Discussion

From the experimental results and the graph it is evident that methylene blue can be used as a tool for study of spoilage in fish tissues. Reay and Shewan[2] in their review on fish spoilage have remarked: "It is possible that a suitable dye or dyes may be indicated. No success has, however, been achieved in this direction."

The results submitted herewith on the use of methylene blue is a partial success to the fulfilment of the above possibility.

While interpreting the above results one should keep in view that the glycolytic breakdown of glycogen to pyruvic acid or lactic acid involves the participation of one dehydrogenase system for which one pair of hydrogen ions is removed from the metabolite and the utilisation of the pyruvic acid to CO_2 by Kreb's cycle involves the participation of two dehydrogenases and three oxidases (functioning by electron transfer) for which five pairs of hydrogen ions are released. Thus when both the processes operate simultaneously the rate of dehydrogenase activity will be very high and consequently there will be less accumulation of acid. This is the position with respect to spoilage of some non-air-breathing species of fish like Chapila, Puti etc. during storage and is represented by the upper part of the broken curve of the graph. The tendency for slope of the curve at higher values would account for a limit of the utilisation of acid at which the enzyme activity would behave in this way. If only the Kreb's cycle system operates, there will be comparatively lesser activity than above and also very small quantity of acid will accumulate. But if the glycolytic system only operates, the tissues will then show very low amount of enzyme activity and so comparatively greater amount of acid will accumulate in the tissues. This second process is represented by the lower part of the broken curve and seems to be manifested by the air-breathing species like Koi, Singi etc. on their storage. In this respect the other fish may behave between the above two extremes.

An interesting correlation between the utilisation of acid, i.e., fall in titratable acidity for an individual fish after 24 hours storage, and the corresponding values of dehydrogenase activities is further evaluated by the data in the last column of Table I, which denotes the product of D, the dehydrogenase activity, and F, the fall in titratable acidity. From these data and also from the graphical representation in Fig. 1, it is noted that the values of the product initially show a rise and thereafter almost a constancy with the increase in the values of F. This curve has a saturation value like that of enzyme activity substrate relationship hypothesised by Michaelis and Menten.[9] Its interpretation however requires further experiments, which are in hand.

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STUDIES ON CITRULLUS COLOCYNTHIS SCHRAD.

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(Received October 1, 1959)

Two crystalline sterols have been isolated from the roots of *Citrullus colocynthis* Schrad., which differ from those isolated so far, and have been tentatively named citrullus colocynthis sterols A and B. Isolation of the alkaloid colocynthis in a crystaline form could not be achieved.

Citrullus colocynthis Schrad., locally known as 'indrayan' or 'tumbi', grows wild in the sandy lands of the north-west, the Punjab, Sind, central and southern parts of Pakistan and India. No where in the subcontinent is it systematically grown. The plant finds mention in the Indian Pharmaceutical Codex 1953 and also in Nadkarni's Indian Materia Medica. According to the latter, all parts of the plants contain traces of an alkaloid and a bitter principle called colocynthin; but since the alkaloid was present only in a very minute quantity, it could not be isolated. It has been mentioned further (under the physiological action of the plant) that in moderate doses it is a drastic hydrogogue, cathartic and diuretic. The physiological properties are attributed to the bitter principle called colocynthin. The different parts of the plant have long been used with advantage in the Indo-Pakistan sub-continent as a drastic purgative in ascites, jaundice and in various uterine conditions.

According to the Indian Pharmaceutical Codex, 1953, page 73, colocynth (dried pulp of the fruit) contains a bitter, amorphous, purgative alkaloid and an amorphous, purgative resin, a small amount of an amorphous glycoside, and the following physiologically inactive substances : α-elaterin, a sterol called citrullol, a hydrocarbon hentriacontane, a phytosterol and a mixture of fatty acids. The seeds contain about 15% of a fixed oil, traces of an alkaloid, an enzyme and a phytosterol.

Even before the start of the present century, sufficient investigation of the plant had already been done by several workers. Among the earliest workers, Walz[1] reported the presence of an amorphous glucoside, colocynthin, $C_{56}H_{84}O_{23}$, and a crystalline substance colocyntnitin, the data of which were not given. Walz was followed by other workers like Henke,[2] and Johannson[3] but nothing notable was reported. Maylor and Chappel[4] succeeded in crystallising the colocynthin as pale yellow needles.

Later, Power and Moore[5] claimed that colocynthin and colocynthetin were not definite individuals. They reported the isolation of the following substances from the pulp: (a) A dihydric alcohol citrullol, $C_{22}H_{36}O_2(OH)_2$, m.p. 285 – 290°C. (no rotation is mentioned), this on acetylation furnishing a diacetyl compound, m.p. 167°C.; (b) an amorphous alkaloid; (c) α-elaterin, proposed formulae : $C_{22-28}H_{28-38}O_{6-7}$, m.p. 233°C.; (d) a hydrocarbon, hentriacontane $C_{13}H_{64}$, m.p. 68°C., optically inactive ; (e) a phytosterol, $C_{27}H_{46}O$, m.p. 160 – 162°C.; and (f) some essential oils and fatty acids (few details given).

Agarwal and Dutta[6] in 1934 confirmed the above work and reported the isolation of the same α-elaterin and hentriacontane from the roots of the plant. Next in sequence are Alim Chandani and Tuman Katti[7] whose investigation led to the isolation of a hydrocarbon, m.p. 59–60°C., and three phytosterols, melting at 122–125°C., 288–290°C., 98–100°C., respectively; rotatory power and analysis were not given. B. Hamilton and W. O. Kermack[8] obtained a sterol, α-spinasterol, m.p. 164–165°C., from the fruits.

Comparatively recently, Rafat Siddiqui, Iqbal Rafat Siddiqui and Sultan Mohammad[9] working on the fruits isolated: (a) a semi-crystalline powder citrulluin, $C_{21}H_{30}O_7$, m.p. 125–127°C.; (b) a micro-crystalline powder citrulluene, $C_{21}H_{32}O_{10}$, m.p. 175–178°C.; and (c) a crystalline acid citrulluic acid, formula not given, m.p. 109°C.

In the present investigation work was taken up on the roots of the plant because they had so far attracted relatively lesser attention. The primary object of the work was the isolation of its alkaloid which had evaded all efforts aimed at crystallisation or crystalline salt formation. In spite of all efforts, this could still not be achieved, but during the course of investigation two sterols could be isolated, which differ from those isolated so far, and have tentatively been named as citrullus colocynthis sterols A and B.

First Processing

In an attempt to obtain the alkaloid, the present authors followed two different courses for

processing. In the first, coarsely crushed roots of *Citrullus colocynthis* Schrad., obtained from Hamdard Dawakhana, were directly extracted with alcohol (six times), when, on removal of the solvent, a dark brown viscous residue could be obtained. Except the inorganic salts, which were found to be mainly sodium and potassium chloride, no crystalline substance was visible in the residue. In accordance with earlier reports, the alcoholic residue, when treated with slightly acidified water and tested for alkaloid, gave a strong positive test. The entire alcoholic residue was treated with glacial acetic acid, when a small amount of a white crystalline residue separated out. From this insoluble residue, the two sterols citrullus colocynthis sterols A and B could be isolated, while the glacial acetic acid solution was treated as follows:—

Attempts to Isolate the Alkaloid.—The glacial acetic acid solution was exhaustively extracted with petroleum ether (60 – 80°C.) when a greenish yellow oil was obtained from the petroleum ether extract while a thick dark-brown jelly-like residue separated out. This residue was poured portion-wise with constant stirring into water, when a light-red aqueous solution showing a positive alkaloidal test was formed, while a semi-solid brown residue separated out, which became a solid powder on rubbing with more water. The brown powder could not be crystallised from any solvent, in most of which it was soluble, and did not contain alkaloid any more. The slightly acidic (pH 5) alkaloidal aqueous solution did not give any copious precipitate with concentrated ammonia, 2N sodium carbonate or 2N sodium hydroxide. There appeared a slight turbidity on addition of sodium hydroxide, which was extracted with ether but gave a negative alkaloidal test.

It was finally decided to subject the water-soluble alkaloidal residue to adsorption analysis on alumina according to Brockmann (E. Merck), using methyl alcohol as eluent. None of the nearly 200 fractions collected contained any crystalline material or could be induced to crystallise. It was not possible to separate the alkaloid even in a concentrated form.

The residue of the red solution, however, did not show any visible salt formation with perchloric acid, flavianic acid, reinecke acid, picric acid, oxalic acid and sulphuric acid. Extensive efforts to isolate a crystalline substance from this brown brittle residue by the process of fractional crystallisation, employing the usual organic solvents, also failed.

Treated with 2N sulphuric acid, the residue of the alcoholic extract behaved in the same way as with acetic acid except that a darker red solution was obtained besides the brown paste. On adding concentrated ammonia to the sulphuric acid solution, a precipitate in the form of an emulsified oil appeared at pH 2-3, which went into solution on addition of more ammonia; the solution still remained slightly acidic. The oil went in solution with sulphuric acid as well as with ammonia, thus showing its amphoteric character.

Similarly the 2N sodium carbonate extract of the residue of the alcoholic extract gave a turbidity on acidification with 2N sulphuric acid and also a positive alkaloidal test. 2N Sodium hydroxide, however, dissolved the residue of the alcoholic extract much quicker and without leaving any appreciable residue at all, thus indicating the phenolic nature of the residue. A phenolic resin seems, therefore, to be present besides an amphoteric alkaloid.

Esterification.—Based on the above assumption, it was thought feasible to esterify the alcoholic residue with methanol and hydrochloric acid gas to convert the amphoteric alkaloid into a base. A black reaction product, from which nothing could be isolated, indicated a sensitivity against strong acids. The esterification was therefore repeated with diazomethane and applied on the residue of the aqueous extract after making it free from the above-mentioned phenolic resin. A gas evolution proceeded smoothly, but the dark viscous residue could not be recrystallised from any solvent. Being still soluble in water, no precipitate with alkali could be expected and was also not observed.

The residue was therefore dissolved in 2N acetic acid and precipitated with potassium bismuth iodide reagent. The orange-coloured complex was filtered, dried and decomposed with hydrogen sulphide in moist acetone solution. The clear light-coloured acetone solution was brought to dryness in vacuum. A very hygroscopic residue mixed with inorganic salts was obtained. Half of the amount was passed through a charcoal column (neutral), while the other half was treated with an ion-exchange resin (Biodeminrolet from Permutit, England).

Charcoal Column.—The concentrated aqueous solution was passed through the column, when only inorganic salts were obtained. The alkaloidal part seemed to be adsorbed. After being freed from inorganic salts, alcohol brought out a yellow non-crystalline substance which gave a positive alkaloidal test. This could neither be crystallised nor was there any salt formation possible with picric, flavianic, Reinecke and oxalic acid.

Ion-exchange Resin.—The aqueous solution after treatment with resin was found to be free of inorganic salts and showed a positive alkaloidal test, but here also all attempts to get something definite failed.

The decomposition of the potassium bismuth iodide complex with silver hydroxide furnished an alkaloid-containing mass in good yield, but again this could neither be crystallised nor could it be converted into salts with the help of acids.

Isolation of Citrullus Colocynthis Sterol A.—The white residue obtained by dissolving the alcoholic extract in glacial acetic acid could, on treatment with ethyl acetate, be separated into a soluble and insoluble fraction. From the soluble fraction, citrullus colocynthis sterol A was isolated as nice colourless needles. Three recrystallisations from a mixture of ethyl acetate and methyl alcohol yielded a pure substance melting at 153–154°C., and showing no rotation in 1% chloroform solution; found C, 83.91; H, 11.68; O, 4.72; mol. wt., 343 (Rast camphor). This fits within the range $C_{22-26}H_{36-44}O$; mol. wt., 316.51 – 372.60. The infrared spectrogram (Fig. 1) showed a strong band between 3000 – 3500 $cm.^{-1}$ suggesting a hydroxyl group. Both the digitonin and Lieberman-Buchardt tests were positive, indicating it to be a 3-β-hydroxy steroid, carrying a double bond.

In order to confirm the presence of the hydroxyl group, the sterol A was acetylated. The acetyl compound recrystallised from alcohol as colourless needles, m.p. 134–135°C.

Isolation of Citrullus Colocynthis Sterol B.—The ethyl acetate insoluble fraction was exhaustively washed with water; the water washings were found to contain merely inorganic salts. The residue was recrystallised from alcohol as colourless plates, m.p. 265 –266°C. (with decomposition). It showed a rotation of $[\alpha]\ ^{32}_{D} = -37.89°$ in 1% pyridine solution. The infrared spectrogram showed a strong band between 3000–3500 $cm.^{-1}$. Liebermann-Buchardt test was positive, while the digitonin test was negative, suggesting it not to be 3-β-hydroxy sterol.

Found: C, 72.59; H, 9.99; O, 17.12. This fits within the range $C_{22-23}H_{36-40}O_4$, m.w., 364.1 – 380.55. The presence of four oxygen atoms in the substance would possibly make it a suitable starting material for the synthesis of cortisone. Such an attempt, however, could not be undertaken because of the relatively small yield of the sterol.

On boiling with acetic anhydride and a few drops of pyridine, two acetyl groups entered the molecule, indicating that there are at least two hydroxyl groups susceptible to acetylation under these conditions. The infrared spectrogram in between 1500 to 2000 $cm.^{-1}$ gave no indication of any carbonyl or ester grouping. Also, there was no band round about 1100 $cm.^{-1}$ indicative of a C-O-C band. Therefore nothing can be said about the function of the remaining two oxygen atoms so far. Surprisingly, on de-acetylation the starting sterol was received back with its unchanged rotatory power, $[\alpha]^{28}_{D} = -33.9°$ in 1% pyridine solution, while the acetyl derivative showed no rotation.

It may be mentioned that citrullus colocynthis sterol B has some similarity with citrullol of Power and Moore in so far as this substance also furnishes a formula of $C_{22}H_{38}O_4$; from which only two oxygen atoms seem to belong to hydroxyl group susceptible to acetylation. The m.p.'s however differ and, while Power and Moore could recrystallise their substance from pyridine with a m.p. of 285–290°C. (with decomposition), the

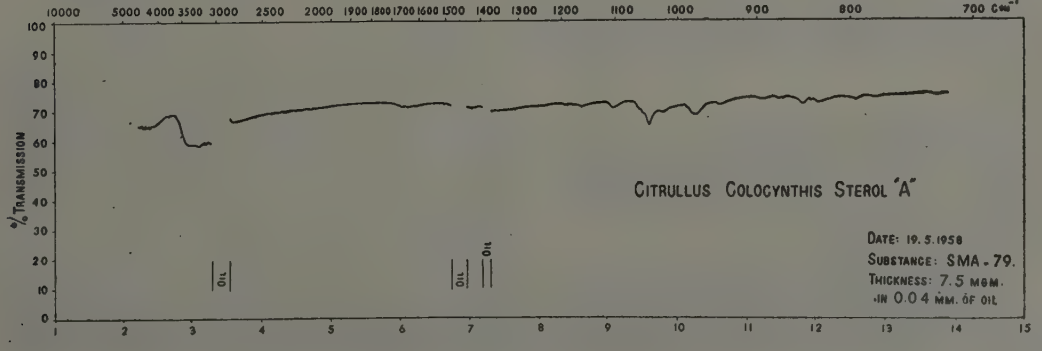

Fig. 1.—The infrared spectrogram of citrullus colocynthis sterol A.

present authors tried in vain to purify their substance from pyridine. After three crystallisations, the m.p. dropped down from 258°C. of the starting raw material to something like 160°C. However, it was found easier to get a pure substance from an alcohol solution. The m.p. of 265 – 266°C. as well as the rotation of $[\alpha]_D^{32} = -37.89°$ remained constant on repeated recrystallisations. The results of analysis were, however, identical with those obtained by Power and Moore.

Second Processing

The coarsely crushed plant roots were first extracted exhaustively with petroleum ether (60 – 80°C.). On removing the solvent, a dark greenish oily mass was obtained, which started depositing a crystalline material on standing. The oily mass was subjected to adsorption analysis on alumina, Brockmann (E. Merck), using petroleum ether, ethyl acetate, alcohol and ether as eluents. Preliminary tests made it possible to combine the 200 fractions collected into seven different groups.

Isolation of Citrullus Colocynthis Sterol A.—The group VI consisted mainly of a crystalline substance. Solubilities and melting point showed it to be identical with citrullus colocynthis sterol A, which had already been isolated during first investigation.

The group No. I, which was mainly an oil mixed with a small amount of a crystalline material, was separated from it by means of adsorption on alumina. The oily parts were distilled under high vacuum. Of the six fractions received, the first two were pure oils while the rest were pasty masses. Fraction V could be sublimed, when four further fractions were obtained. Fraction III was recrystallised from alcohol and found to be citrullus colocynthis sterol A.

It may be mentioned in this connection that only a little more than half of the total petroleum ether extract could be recovered from the alumina column, thus indicating a possible presence of free acids.

Alcoholic Extraction. Isolation of Citrullus Colocynthis Sterol B.—The alcoholic extract on concentration and standing deposited a white crystalline material, which, besides containing a good amount of inorganic salts, contained water-insoluble citrullus colocynthis sterol B.

The residue of the mother liquors, recovered after the removal of citrullus colocynthis sterol B, was recrystallised from alcohol and found to be citrullus colocynthis sterol A.

Experimental

First Method: Extraction with Alcohol

Fresh roots of *Citrullus colocynthis* Schrad. were coarsely powdered in a ball mill as much as possible and extracted with alcohol.

Experiment 1.—1800 g. of the fresh roots were extracted with 34.5 litres of alcohol at room temperature of 32°C., and 119.5 g. of solvent free dark brownish residue (6.6% of the fresh root) was obtained.

Extraction	Time	Solvent	Residue
1	4-7 days	6 litres	67.47 g.
2	,, ,,	6 ,,	26.8 ,,
3	,, ,,	6 ,,	15.4 ,,
4	,, ,,	5½ ,,	2.4 ,,
5	,, ,,	5½ ,,	4.0 ,,
6	,, ,,	5½ ,,	3.5 ,,
		Total =	119.5 ,,

Experiment 2.—Another 1800 g. of fresh roots were extracted with 36 litres of alcohol and 126.3 g. of the solvent free residue (7.01% of the root) was obtained.

Extraction	Time	Solvent	Residue
1	4-7 days	6 litres	73.2 g.
2	,, ,,	6 ,,	31.2 ,,
3	,, ,,	6 ,,	12.0 ,,
4	,, ,,	6 ,,	4.2 ,,
5	,, ,,	6 ,,	3.7 ,,
6	,, ,,	6 ,,	2.0 ,,
		Total =	126.3 ,,

Isolation of Citrullus Colocynthis Sterol A.—63 g. of the alcoholic residue from first extract was treated with nearly 30 to 35 ml. of glacial acetic acid. The major portion of the residue went into solution while a small amount of a white substance (2.644 g.) remained insoluble. Treated with ethyl acetate, this substance could be divided into a soluble (0.837 g.) and an insoluble part (1.807 g.)

The ethyl acetate soluble fraction on recrystallisations (3 times) from a mixture of ethyl acetate and methyl alcohol (2:1) yielded 0.175 g. of a nice colourless crystalline substance (0.277%

of the first alcoholic residue). The m.p. was found to be sharp at 153–154°C. It showed no optical rotation in 1% chloroform solution. Found: C, 83.91; H, 11.68; O, 4.72.3; m.w. (Rast), 342. This fits within the range $C_{22-26}H_{36-44}O$(m.w. 316.51–372.60). Digitonin and Liebermann-Buchardt test were positive. The infrared spectrogram showed the presence of an OH group (shown in Fig.1). It may be named 'citrullus colocynthis sterol A' as long as it cannot be identified with any known sterol.

The substance was readily soluble in chloroform, moderately so in alcohol, acetone, benzene, petroleum ether, ethyl acetate and only sparingly so in methyl alcohol. The quantity of citrullus colocynthis sterol A sharply declined in the subsequent alcoholic extracts.

Acetylation of Citrullus Colocynthis Sterol A.—180 mg. of the sterol A was shaken in a 10 ml. flask with 0.3 ml. of acetic anhydride and a few drops of pyridine for five hours. The crude mass obtained on standing overnight weighed 0.144 g. It was recrystallised from a mixture of ethyl acetate and methyl alcohol as colourless crystals. Yield, 0.0876g.; m.p. 134–135°C. This also showed no rotation in 1% chloroform and was readily soluble in ethyl acetate, chloroform, carbon tetrachloride, moderately so in benzene, acetone, petroleum ether, alcohol and only sparingly in methyl alcohol.

Isolation of Citrullus Colocynthis Sterol B.—1.8 g. of the ethyl acetate insoluble fraction was extracted exhaustively with water until the washings gave no precipitate with silver nitrate. The water washings were brought to dryness and found to contain sodium chloride and potassium chloride. 0.3238 g. of the substance remained insoluble in water. The quantity decreased in later alcoholic extracts.

In order to get more material, the rest of the alcoholic extract was treated likewise when 1.3 g. of the crude ethyl acetate and water insoluble substance was obtained. On recrystallisation from alcohol, wherein it was sparingly soluble, colourless plates weighing 0.548 g. were obtained. After 3 more recrystallisations the m.p. remained constant at 265–266°C. (with dec.) the rotation in 1% pyridine was $[\alpha]^{32}_{D} = -37.89°$. Found: C, 72.59; H, 9.99; O, 17.12. This fits within the range $C_{22-23}H_{36-40}O_4$, m.w., 364.1–380.55. The infrared spectrogram indicated the presence of OH groups (shown in Fig. 2). This sterol may be called 'citrullus colocynthis sterol B.' It gives a positive Liebermann-Buchardt test but a negative digitonin test.

It was readily soluble in pyridine, tetrahydrofuran, dimethylformamide, moderately so in benzene, carbon tetrachloride and only sparingly in alcohol and acetone. It was insoluble in water, ethyl acetate and petroleum ether.

Acetyl Compound of Citrullus Colocynthis Sterol B.—0.373 g. of sterol B together with 4 ml. of acetic anhydride and a few drops of pyridine were refluxed for five hours in a 25 ml. flask. On leaving overnight the acetyl compound crystallised out. It was filtered on mild suction. Yield, 0.1543 g. crude. On several recrystallisations from alcohol, 0.1543 g. of colourless finely crystalline substance was obtained. The m.p. was constant at 159 – 160°C. It showed no rotation in 1% chloroform solution.

It was easily soluble in acetone, benzene, chloroform, ethyl acetate, dimethylformamide, moderately so in alcohol and sparingly in petroleum ether. The report of analysis showed that the acetyl sterol B contained 20.7 % of ($CO.CH_3$) group which would agree with the presence of

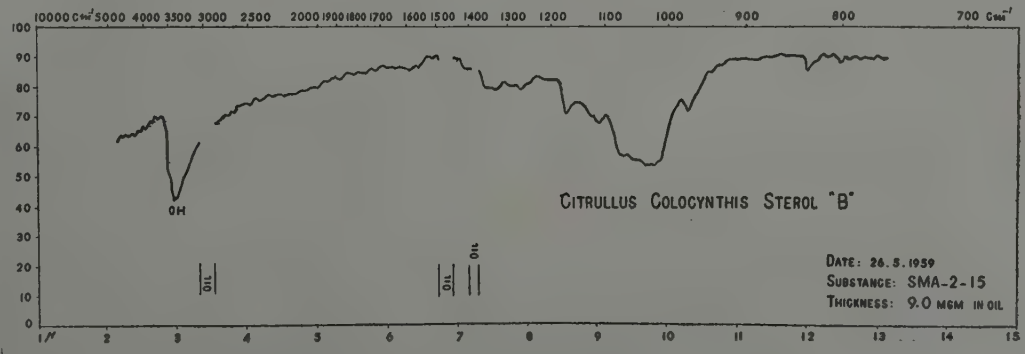

Fig. 2.—The infrared spectrogram of citrullus colocynthis sterol B.

2 hydroxyl groups in its molecule susceptible to acetylation under these conditions. Calculated: $C_{22}H_{34}O_2(O\text{-}COCH_3)_2$, (19.1%),

Deacetylation of Colocynthis Sterol B.—100 mg. of acetyl sterol B was refluxed on a water bath for 5 hours with 60 mg. of potassium hydroxide in alcohol solution and left overnight. The alcohol was removed and the residue treated with water, filtered from the insoluble which was again washed with water until it gave no alkaline test with litmus. The substance was recrystallised from alcohol. Yield, 0.0195 g. The deacetylated compound showed a rotation of $[\alpha]_D^{28} = -33.9°$ in 1% pyridine, which agrees with that of the original sterol B. It melts at 265 – 266°C. Solubilities were similar to the original sterol B.

Precipitation of an Alkaloid.—47.5 g. of the alcoholic residue was shaken three times for a total period of an hour with 50 ml. of water each, slightly acidified with acetic acid. The solution was always decanted off from a brown viscous water-insoluble resin. The third extract gave only a weak test with potassium bismuth iodide reagent. The residual oily resin weighed 20.73 g. after drying over phosphorous pentoxide at room temperature.

The three combined extracts were precipitated with potassium bismuth iodide reagent and the resulting orange coloured substance filtered by suction, washed with water, and dried on a porous plate. Yield, 18.2048 g. or 38% of the alcoholic residue.

Potassium bismuth iodide complex (1.6 g.) was shaken for 5 hours in a 250 ml. glass stoppered flask containing a few glass beads and 3 g. of freshly prepared silver hydroxide and 100 ml. of acetone. It was filtered and the filtrate treated a second time with a fresh quantity of silver hydroxide in the same way, as it still showed some colour, filtered and the filtrate brought to dryness. Yield, 0.2303 g., i.e., 14% of the complex of 5% of the alcoholic residue.

The black residue in the flask was again shaken with another 100 ml. of acetone for 5 hours, filtered and the solvent removed. Yield, 0.0082 g.

The light brownish powder (0.2303 g.) was found to be very sparingly soluble in water more easily in concentrated hydrochloric acid, from where it was precipitated out on dilution. There was no visible salt formation with flavianic acid, picric acid, oxalic acid and sulphuric acid. It, however, gave a thick precipitate with usual alkaloidal reagents in acidic solutions.

Second Method: Extraction with Petroleum Ether

Experiment 1.—2 kg. of coarsely powdered roots of *Citrullus colocynthis* Schrad. were extracted six times with petroleum ether (b.p. 60 – 80°C.) at room temperature (32°C.) as follows:

Extraction	Time	Solvent	Residue
1	24 hours	8 litres	5.6017 g.
2	48 ,,	8 ,,	2.5410 ,,
3	48 ,,	7½ ,,	1.0890 ,,
4	48 ,,	7½ ,,	0.6045 ,,
5	48 ,,	7½ ,,	0.6297 ,,
6	48 ,,	7½ ,,	0.3713 ,,
		Total =	10.8372 ,,

Solvent-free green oily residue, 10.8372 g. (0.541% of the root) was obtained, which on standing for several days deposited increasing amount of a crystalline material.

Experiment 2.—Another 2 kg. of roots were extracted with a total of 49 litres of petroleum ether (b. p. 60 – 80°C) by percolation at room temperature (32°C), according to the following scheme.

Extraction	Time	Solvent	Residue
1	48 hours	8½ litres	5.8360 g.
2	48 ,,	8 ,,	2.2265 ,,
3	48 ,,	8 ,,	1.2060 ,,
4	48 ,,	8 ,,	0.5746 ,,
5	48 ,,	8 ,,	0.3250 ,,
6	48 ,,	8 ,,	0.2670 ,,
		Total =	10.4351 ,,

The yield was 10.43 g. of green oily residue (0.521% of the root). A further quantity of the roots was extracted with petroleum ether in the same way as described above when a total of 53.2 g. of the substance was collected.

Adsorption Analysis of the Petroleum Ether Extract on Alumina (Brockmann, E. Merck).—53.2 g. of the green oily mass containing the crystalline material was dissolved in a minimum quantity of petroleum ether (b.p. 60 – 80°C.), placed on top of a column of 50 cm. length, 3½ cm. width, filled with 279 g. of alumina made wet with 300 ml. of petroleum

ether. Elution was done successively with petroleum ether, ethyl acetate, benzene, alcohol and ether.

A total of 216 fractions were collected taking 50 ml. and later 100 ml. each time. Based on the physical appearance, solubilities and microscopic examination, the collected fractions could be combined into seven different groups as shown in the following table:

Fraction No.	Eluent	Eluent used ml.	Amount in g.	Group No.	Remarks
1- 10	Petroleum ether	50	18.4067	I	Light green oil with small amount of a crystalline material
11- 20	,,	50	2.2026	II	Colourless needles mixed with pasty mass
21- 26	,,	50	0.4170	III	Broad rod shaped crystals with a pasty mass
27- 35	,,	100	0.4744	IV	Substance containing few rods but mostly clusters
36- 62	,,	100	0.6771	V	Mainly cluster-shaped substance with pasty mass
63-150	,,	100	3.2810	VI	Beautiful needles of a colourless crystalline substance
151-190	Ethyl acetate	100	41,160	VII	Light yellow uniform oil
691-207	Alcohol	100	1.8439		
208-216	Ether		0.5398		
		Total ..	31.8458		
		Balance ..	21.4 g. remained in column.		

Investigation of Group VI. Isolation of Citrullus colocynthis Sterol A.—3.28 g. of the crude mass obtained from group VI (fractions 63 – 150) was recrystallised from alcohol. M.p. 153 – 154°C, yield 0.1315 g. It was found to be identical with citrullus colocynthis sterol A (described under first method).

Investigation of Group I.—17.59 g. of the oil mixed with a small amount of crystalline substance from group I (fractions 1–10) was dissolved in a minimum quantity of petroleum ether and passed through a column of alumina according to Brockmann (E. Merck) of 51 cm. length and 2 cm. width containing 120 g. of alumina. Elution was done with petroleum ether and later with ethyl acetate. Fractions of 50 ml. each were collected and the solvent removed. A total of 14.527 g. of a light yellow oil could be recovered in this way which appeared to be uniform in appearance, 3.0 g. of oil remaining in the column. Benzene and chloroform failed to bring down any substance. Twenty oil fractions collected in the way described were mixed together and distilled in high vacuum from a copper block.[10]

The following fractions were obtained.

Fraction	Amount	Temperature	Pressure	Remarks
1	2.0472 g.	130–160°C.	0.06 mm.	Light-coloured oil
2	2.1440 g.	106–190°C.	0.06 mm.	Light-coloured oil
3	0.8945 g.	190–230°C.	0.06 mm.	Mostly oil with a solid substance
4	0.1550 g.	230°C.	0.06 mm.	Solid substance with a little oil
5	0.7660 g.	230–160°C.	0.06 mm.	Solid material with little oil
6	1.4680 g.	260–290°C.	0.06 mm.	Solid mixed with oil (could not be crystallised or sublimed)
Total =	7.4747 g.			

The light-coloured oil from fractions 1 and 2 (339 mg.) was saponified, when a coloured oily alcohol (0.198 g.) and a fatty acid 0.230 g. were obtained. The fatty acid was found to give a urea inclusion compound. It was dried thoroughly over phosphorus pentoxide and then treated with 0.15 ml. of phenyl isocyanate for 5 hours, at 180–185°C. The excess of isocyanate was removed in vacuum. Residue, 0.370 g. (oily mass). In spite of all efforts, the anilide could not be obtained in a pure state due to the small amount. 0.766 g. of the pasty mass from fraction 5 could not be recrystallised from any solvent and so was sublimed under high vacuum.

Four fractions were collected as shown:

Fraction	Amount	Temperature	Pressure	Remarks
1	0.2081 g.	upto 150°C.	0.06 mm.	Pasty mass
2	0.0594 g.	upto 160°C.	0.06 mm.	Pasty mass
3	0.3219 g.	upto 230°C.	0.06 mm.	White solid substance
4	0.0124 g.	upto 270°C.	0.06mm.	Pasty mass
Total =	0.6018 g.			

Fraction No. 3, 0.321 g. of the white substance, was recrystallised from alcohol. Yield 0.0573 g., m.p. 153 – 154°C. The solubilities, crystal shape and m.p. were identical with citrullus colocynthis sterol A (described under first method). All attempts to get crystalline material from the other fractions failed.

Investigation of groups II to VII did not lead to any pure substance.

Extraction with Alcohol.—2 kg. of roots after exhaustive extraction with petroleum ether were extracted with a total of 53 litres of alcohol at room temperature (31°C):

Extraction	Time	Solvent	Amount
1	48 hours	8 litres	33.3 g.
2	48 ,,	8 ,,	28.8 ,,
3	48 ,,	8 ,,	26.0 ,,
4	48 ,,	7½ ,,	20.5 ,,
5	48 ,,	7½ ,,	12.3 ,,
6	48 ,,	7½ ,,	9.1 ,,
7	48 ,,	7 ,,	3.0 ,,
		Total =	132.0 ,,

i.e., 6.6% of the root (dark brown pasty mass)

Isolation of Citrullus Colocynthis Sterol B.—The first alcoholic extract on concentration and standing deposited 1.0921 g. of a white crystalline material. It was filtered and repeatedly washed with water in order to remove the inorganic salts specially sodium and potassium chloride until the washings did not give any precipitate with silver nitrate.

The white residue was recrystallised (three times) from alcohol in which it was sparingly soluble. Yield 0.2765 g., m.p. 265–266°C. On the basis of m.p. and solubilities it was identified as citrullus colocynthis sterol B (described under first method).

Acknowledgement

Microanalysis reported was carried out by A. Bernhardt. Mikroanalytisches Laboratorium in Max-Planck institut, Muelheim/Ruhr, West Germany.

The authors are indebted to Hakim Mohammad Said of Hamdard Dawakhana for the supply of roots of *Citrullus colocynthis* Schrad. used in this work and also to M.M. Qurashi of Physics Division of the Central Laboratories for carrying out the infrared spectrography.

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MEAN FREE PATH OF 4.5 BEV π–MESONS IN ILFORD G5 EMULSIONS*

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(Received October 1959),

The experimental value of the mean free path for the nuclear interaction of artificially produced π-mesons of 4.5 Bev. energy has been found to be 33.5±3.5 cm. Ilford G-5 emulsions. 400 microns thick, were exposed to the high energy meson beam, the measurements being made by the so-called "Line Scanning" or "the following along the track" method. Our result is comparable with earlier data for mesons with energies upto 3 Bev.

Introduction

Much progress was made in our knowledge of the properties of π-mesons since their discovery in 1947. Many facts were first established by cosmic ray experiments. The possibility of artificially producing π-mesons in large numbers has opened the field to more refined means of experimentation. These have given us precise informations on many properties of these mesons which are at least qualitatively understood on a theoretical basis.

Experiments on the nuclear interaction of artificially produced π-mesons have been carried out at several laboratories, viz., Columbia, Rochester, Berkeley and Chicago. But it appears that there exists definite disagreement between the experimental resluts obtained at these laboratories. Since the study of the interactions between π-mesons and nuclei are considered to be so important for a clear understanding of the problem of nuclear forces, it was felt desirable to carry out further investigations on the problem. The main object of the present work has been to obtain the experimental value of the mean free path for this interaction using π-mesons of a higher energy, viz. 4.5 Bev.

Experimental Technique

(*i*) *Exposure of Plates.*—Ilford G-5 emulsions, 400 microns thick, were exposed to the high energy meson beam of the Berkeley Bevatron. The beam with an average energy of 4.5 Bev. was allowed to hit the end of the stack of emulsions in a strip 3″ wide in the centre of the stack. The negative pions were selected by an analysing magnet from the secondary particles emitted from a target at 90° to the direction of the proton beam. The emulsions were placed with the 3″ side parallel to the pion beam.

(*ii*) *Microscopic Measurements.*—Measurements were made by the so-called "Line Scanning" or "the following along the track" method. The plate was placed on the microscope stage in such a way that the π-meson tracks were all parallel to the X-axis, so that the X-motion of the stage could be used to measure the length of each track scanned. The beginning of each track was made coincident with some point on one side of the rectangular mesh, that is, the field in view, and the track was followed by advancing the X-motion of the stage in steps and varying the depth of focus until the end of the track was reached. The distance between the two readings at the beginning and the end as read on the vernier scale of the microscope stage gives the length of the track scanned. The stage was then brought back to the original point of starting and was then moved in the Y-direction to the next field of view and the tracks were followed as before. The operations were repeated until a sufficient length of the tracks was measured.

It was observed that tracks occasionally collided with emulsion atoms and produced nuclear disintegrations, more popularly termed as "Stars". The magnification employed all through in the present investigation was 45 × 12, that is, a X45 objective was used in conjunction with a X12 eyepiece.

Since all the π-tracks were approximately flat, the angle of dip was not taken into account.

Experimental Results and Conclusions

In scanning a total length of 32.85 metres of π-meson tracks, 98 interactions were found. All these interactions were nuclear disintegrations of emulsion nuclei initiated by the 4.5 Bev. π-mesons.

No event of elastic scattering or disappearance in flight was observed. Hence the mean free path for nuclear interaction was found to be 33.5±3.5 cm. The ratio of the geometrical to the observed mean free path was thus obtained as 1.34.

From Table 1, in which the known data for π-mesons of various energies are collected, it can

* The experimental part of the work was done while the authors were in the Physics Department, University of Dacca, Dacca.

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TABLE 1.—MEAN FREE PATH OF π--MESONS IN ILFORD G5 EMULSIONS.

Energy range	M.F.P.	Observed value/ Geom. value	Authors
70- 80 Mev.	20 ±2.6 cm. of emulsion	1.00	Bernadini et al.
60- 90 Mev.	22.1±4.4 ,, ,,	1.10	,, ,,
100-110 Mev.	18.5±2.0 ,, ,,	0.90	,, ,,
30- 40 Mev.	22.1±2.7 ,, ,,	0.93	Bradner & Rankin
210 Mev.	25.7±1.6 ,, ,,	1.03	Morrish
750 Mev.	38.5±2.2 ,, ,,	1.54	Blau & Oliver
3 Bev.	35.5±5 ,, ,,	1.42	Schien et al.
4.5 Bev.	33.5±3.5 ,, ,,	1.34	Present work

be seen that the mean free path is very nearly geometrical and remains constant in the energy range 30-210 Mev. However, it is found that it rises to a value of 38.5±3.2 cm. at 750 Mev. as observed by Blau and Oliver, while Schien, Haskin and Glasser obtained a value of 35.5±5 cm. for 3 Bev. π-mesons. This compares well with our result, viz., 33.5±3.5 cm. for 4.5 Bev. π-mesons, making allowance for the experimental and statistical errors involved in the measurements. Thus it can be seen that at very high energies the mean free path of π-mesons in Ilford G5 nuclear emulsions remains constant at about 36 cm., which is significantly higher than the geometrical value, viz., 25 cm.

Acknowledgements

The authors acknowledge their grateful thanks to Professor C.F. Powell of the University of Bristol, England, for kindly lending the plates on which measurements were made. The plates were exposed to pi-mesons at the Lawrence Radiation Laboratory, Univeristy of California, Berkeley, California.

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Note Added in Proof.—It is of interest to add that in a recent experiment with 14 GeV/c π-mesons from the CERN proton synchrotron, the mean free path of pions in emulsion has been found to be 32.8 ± 1.5 cm. (private communication from Lock and Herz, to be published soon in Nuovo Cimento).

THE INFLUENCE OF LONG-CHAIN PHENOLIC ADDITIVES ON THE VISCOSITY OF MINERAL OILS

Part V.—Phenomena occurring in the Proximity of the Miscibility Limit in Mixtures of Allyl Catechol and Mineral Oil

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Earlier experiments on the viscosity depression occurring in solutions of allyl catechol in mineral oil have been extended, and the results are analyzed to investigate the nature of the large anomalous *increase* in viscosity noticed in the range of 30% to 80% allyl catechol. This increase above the normally depressed value has been estimated as a Reduced Viscosity i.e. $(\Delta\nu/\nu) \div$ concentration, and plottted as a function of the temperature excess, ΔT, above the miscibility temperature for four different concentrations of allyl catechol varying from 20% to 70%. From this data, the graph for Intrinsic Viscosity (i.e. the limit of reduced viscosity at zero concentration) as a function of ΔT is obtained, which lends itself to an interpretation of the phenomenon as an incipient separation into macro-molecules of the two components. The Intrinsic Viscosity for $\Delta T \rightarrow o$ is about 0.3, and, by using Staudinger's equation for the degree of polymerization in colloids, it is possible to estimate the number of molecules in each macro-molecule as about 70 when just above the miscibility temperature.

On the basis of these experimental data, an attempt is made to explain the different values of the coefficient of the quantity, $\ln\nu$, in the empirical equation, $\ln(-\Delta\nu) = K + A \ln\nu$, in the case of (i) variation of number of OH groups in a single series of phenolic compounds, and (ii) variations of temperature for a given binary system.

Introduction

Some measurements on the viscosities of mixtures of allyl catechol with mineral oil at various temperatures were presented in an earlier communication,[1] dealing with the development of a law governing the viscosity depression that occurs when a phenolic compound is mixed with a mineral oil blend of the same viscosity. Whereas the curves for the other compounds studied were of the expected character with a (single) minimum of viscosity near the middle, those for allyl catechol showed an anomalous increase in viscosity in the region of 30% to 85% allyl catechol (Fig. 1(a)). The relative magnitude of this anomalous rise was found to increase as the temperature was decreased towards the miscibility temperature (Fig. 1(b)) for the corresponding concentration of allyl catechol.

Since the normal drop in viscosity is approached at temperatures far above the miscibility limit for a particular binary mixture (cf. Figs. 1(a) and 1(b)), it appears that the total change in viscosity

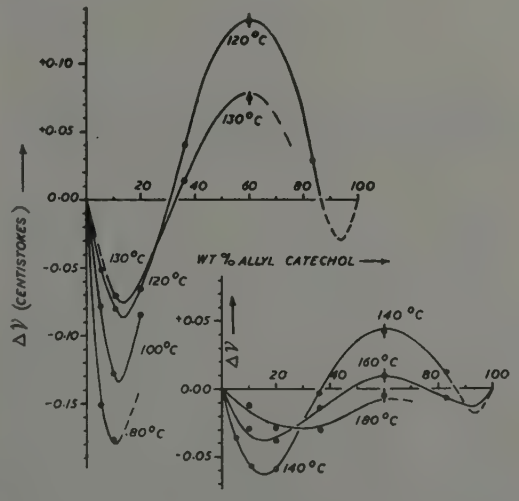

Fig. 1(a).—Experimentally observed viscosity depression and the anomalous viscosity increase in mixtures of allyl catechol and mineral oil at various temperatures from 80°C. to 180°C.

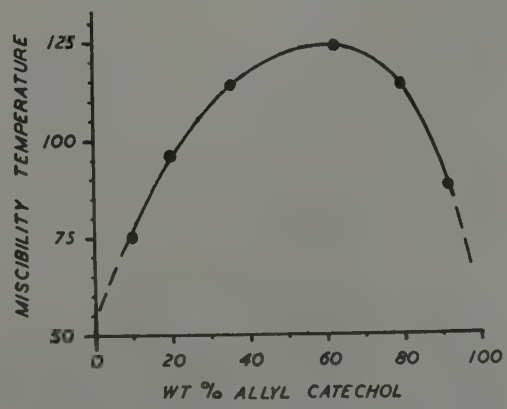

Fig. 1(b).—Phase diagram, showing miscibility temperatures in the allyl catechol-mineral oil system.

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can be represented as the sum of (a) the normal viscosity depression, and (b) the anomalous rise, which depends on the proximity of the miscibility temperature. It was therefore considered of interest to separate these two effects quantitatively, and to study the dependence of the viscosity increase on the concentration of the allyl catechol and on the departure from the miscibility temperature.

Effect of Oxidation of the Allyl Catechol

For quantitative interpretation of the graphs of Fig. 1(a), it is important to note that at the elevated temperatures of these measurements, the viscosities of both the mineral oil blend and the allyl catechol increases continuously with heating, due mainly to loss of the volatile components in the first case and oxidation in the second case. In order to measure this permanent increase (in viscosity) and to estimate its dependence on concentration of the catechol in the mixture of oil and allyl catechol, some of the mixtures were heated two or three times over the range of temperatures used in the experiments of Fig. 1, and the mean percentage increase in viscosity at several temperatures was calculated for one heating. These results are plotted against temperature in Fig. 2(a) and the best lines have been drawn through the points for each concentration, the uppermost line being for 100% allyl catechol. These graphs approximate to a pencil of straight lines meeting at a point somewhat above 200°C., and their spacing indicates that the increase in viscosity at any one temperature is roughly proportional to the concentration of allyl catechol plus a constant. Fig. 2(b) shows plots of this increase as a function of concentration at two different temperatures, namely 100° and 150°C. The plotted points fit rather well on the straight lines drawn (through them) thus confirming that, at any one temperature, the calculated viscosity for an ideal solution of the oil and allyl catechol will still be given by a linear relationship between the viscosities of the two components. Figure 2 indicates that the departure from this linearity would be less than 0.2% at 150°C. and about 0.3% at 100°C., which figures are within the limits of experimental error for the measurements of viscosity.

Since the values of $\Delta\nu$ plotted in Fig. 1 are obtained from the relation $\Delta\nu = (\nu_{expt} - \nu_{ideal})$, where ν_{ideal} has been calculated by a linear interpolation between the measured values of ν_{oil} and $\nu_{allyl\ cat.}$, it follows that no additional correction for the effects of the permanent change in viscosity discussed above is required for these values of $\Delta\nu$.

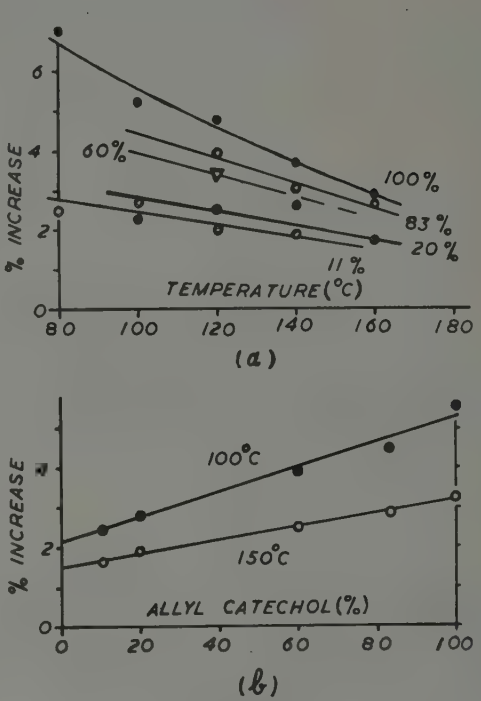

Fig. 2.—Graphs of the permanent viscosity increase due to oxidation in one cycle of heating, measurement and cooling: (a) experimental data plotted against temperature for five different concentrations, and (b) smoothed data plotted (for two temperatures) as a function of concentration to show the linear relationship.

Quantitative Estimation of the Anomalous Rise in Viscosity for Mixtures containing 20% to 70% Allyl Catechol

Since the measurements on allyl catechol mixtures given in the previous communication[1] on this subject were primarily designed to estimate $-(\partial\nu/\partial x)_{x=0}$ no mixtures with allyl catechol concentration between 36% and 82% were used. For the present investigation, however,this range of concentrations is significant and therefore further viscosity measurements were made (in the single phase region) on a mixture containing 60% allyl catechol, and the corresponding values of $\Delta\nu$ are plotted in Fig. 1(a) as solid circles with a short vertical line. The inclusion of this extra set of points makes it possible to draw the complete variation of $\Delta\nu$ quite statisfactorily, even (in some cases) beyond 80% allyl catechol.

In order to determine the magnitude of the anomalous rise in viscosity, it is first necessary to

estimate the normal viscosity depression from the shapes of the curves observed in the other two members of the allyl series. The experimental values of $-(\partial\nu/\partial x)_{x=0}$ for allyl catechol at different temperatures are known from the original slopes of the graphs in Fig. 1(a),* and these enable $\Delta\nu_{max}$ also to be estimated with the help of the known ratios of $\Delta\nu_{max}$ to $(\partial\nu/\partial x)_{x=0}$ tabulated in Table 1 for the phenolic compounds studied in the present series of investigations.

It is clear that a value of $0.14 \pm 0.01_5$ can be used confidently for this ratio in the case of allyl catechol and that the concentration for the normal minimum in the viscosity-concentration curve can be taken as 40% ± 3%. With the help of this data, and using the additional information (last row of Table 1) that $(\partial\nu/\partial x)_{x=100\%} \simeq 0{\cdot}39(-\partial\nu/\partial x)_{x=0}$, the "normal" curves to be expected for allyl catechol can be drawn reasonably accurately, at least upto 70% allyl catechol, as shown by the broken lines in Figs. 3(a), 3(b) and 3(c) where the experimental graphs are indicated by solid lines. The differences, $\delta\nu$, between these experimental graphs and the calculated "normal" curves can be measured, and are given in Table 2, together with the values of the (fractional) specific viscosity increase, $\delta\nu/\nu_{mean}$, for four different concentrations of allyl catechol, namely 20%, 35%, 50% and 70%.

It is seen that the specific viscosity increase is strongly dependent on both the concentration of the allyl catechol and on the proximity to the

*In the calculations, the smoothed values obtained by plotting $(\partial\nu/\partial x)_{x=0}/\nu$ allyl cat. against temperature have been used as being rather more reliable.

TABLE 1.—COMPARISON OF CONSTANTS DETERMINING THE SHAPE OF THE CURVE FOR VISCOSITY DEPRESSION $\Delta\nu$ IN SEVERAL PHENOLIC COMPOUNDS.

Compounds:	Bhilawan series at 40°C.	Bhilawanol at various temperatures	Mean	Allyl series at 40°C.	Allyl phenol at various temperatures	Mean	Overall Mean
$\Delta\nu_{max}/(\partial\nu/\partial x)_{x=0}$	0.14	0.20	0.17±0.03	0.15_5	0.12_5	$0.14\pm0.01_5$	0.15±0.02
$100\ (x)_{max}$	39±4	35±2	37±2	43±3	36±5	40±3	38±2
$-\left(\frac{\partial\nu}{\partial x}\right)_{x=100\%} \div \left(\frac{\partial\nu}{\partial x}\right)_{x=0}$	0.20±0.03	0.45±0.05	0.32±0.1	0.55±0.1	0.35±0.1	0.45±0.05	0.39±0.06

TABLE 2.—MEASURED VALUES OF THE ANOMALOUS INCREASE IN VISCOSITY AND THE CORRESPONDING SPECIFIC VISCOSITY FOR ALLYL CATECHOL-MINERAL OIL MIXTURES AT SEVERAL TEMPERATURES.

Temperature (°C.)		180	160	140	130	120	100	80	Miscibility temperature
Anomalous increase in viscosity	20% allyl cat.	.008	.013	.021	.034	.056	(.115)	(.30)	94°C.
	35% ,, ,,	.017	.050	.091	.128	.182	(.35)	—	112°C.
	50% ,, ,,	.031	.070	.132	.180	.260	—	—	120°C.
$= \delta\nu$ (centistokes)	70% ,, ,,	.026	.052	.108	.152	.215	—	—	120°C.
ν_{mean} = mean viscosity of oil & allyl catechol		0.84	1.01	1.26	1.43	1.65	2.31	3.52	
Specific viscosity increase	20% allyl cat.	.009	.013	.017	.024	.034	.050	(.085)	94°C.
$=\delta\nu/\nu_{mean}$	35% ,, ,,	.020	.050	.072	.090	.110	(.15)	—	112°C.
	50% ,, ,,	.037	.069	.105	.126	.158	—	—	120°C.
	70% ,, ,,	.031	.051	.086	.106	.130	—	—	120°C.

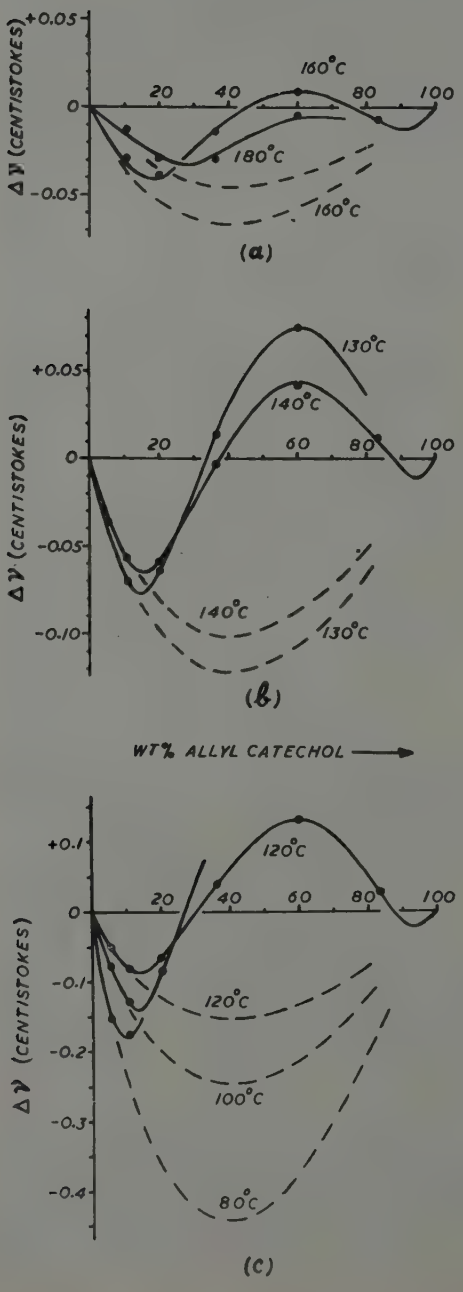

Fig. 3.—Curves for the quantitative estimation of the anomalous viscosity increase above the normal depression. Broken lines, calculated "normal" curves; solid lines, experimental graphs of Fig. 1(a): the differences between corresponding solid and broken lines give the anomalous increase.

miscibility temperature for the particular concentration. When allowance is made for the difference in miscibility temperatures for the four concentrations, it appears that, for concentrations below 50% allyl catechol, the specific viscosity $\delta\nu/\nu_{mean}$ is roughly proportional to the concentration, reaching a maximum at about 50% catechol, after which it decreases with increasing catechol concentration. This behaviour can to some extent be compared with the viscosity of a dilute suspension of small particles in a liquid, for which the specific viscosity is proportional to the concentration of these small particles, as given by Einstein's equation.[2] An explanation of the anomaly was therefore sought along these lines.

Interpretation in Terms of Einstein's Equation for Suspensions

For the case of solid spherical particles, the theoretical relation developed by Einstein[2] and other workers[3] for the relative viscosity η/η_0 is

$$\eta/\eta_0 = 1 + 2.5 \times (\text{Vol. concentration}) + \alpha\,(\text{Vol. Conc.})^2, \quad (1)$$

$$\text{i.e. Specific Viscosity} = (\eta-\eta_0)/\eta_0 = \delta\eta/\eta_0 = 2.5\,(\text{Vol. Conc.}) + \alpha\,(\text{Vol. Conc.})^2 \quad (2)$$

$$\text{whence Reduced Viscosity} = (\delta\eta/\eta_0)/\text{Conc.} = 2.5 + \alpha\,(\text{Vol. Conc.}). \quad (3)$$

The above relations hold for (volume) concentrations of much less than 50%. If now we postulate that, at temperatures only slightly above the miscibility temperature for a given concentration of allyl catechol, the molecular aggregates of the catechol behave to some extent like a suspension of very small particles in the oil, then equation (3) may be assumed to apply, albeit with a smaller value of the constant than 2.5, because the "particles" in this case are not *solid* spheres. The experimental value of this constant should be expected to increase rapidly as the miscibility temperature is approached. In order to test these ideas, the Reduced Viscosity has been calculated as $\{(\delta\nu/\nu_{mean})/\text{concentration}\}$ in Table 3, $\delta\nu/\nu$ and concentrations by weight being taken as a sufficiently good approximation to $\delta\eta/\eta$ and volume concetrations, respectively, because of the small difference in density.

The reduced viscosity for any particular temperature excess above the miscibility temperature

TABLE 3.—THE REDUCED VISCOSITY OF ALLYL CATECHOL – MINERAL OIL MIXTURES AT DIFFERENT TEMPERATURES.

Temperature (°C.)		180	160	140	130	120	100	80	Miscibility temperature
Reduced Viscosity $= \left\{ \frac{\delta\nu/\nu_{mean}}{\text{Concentration}} \right\}$	20% allyl cat.	.045	.065	.085	.120	.170	.25	(.42)	94°C.
	35% „ „	.057	.143	.206	.257	.315	(.44)	—	112°C.
	50% „ „	.074	.138	.210	.252	.316	—	—	120°C.
	70% „ „	.044	.073	.123	.151	.186	—	—	120°C.
	(100-30)% „	.103	.170	.287	.353	.433	—	—	120°C.

is seen to increase slowly with concentration upto nearly 50% allyl catechol, so that it would tend to a definite non-zero limit (the Intrinsic Viscosity) for small concentrations. In order to determine this limit for various temperatures above the miscibility limit, the following procedure was used:

(a) The reduced viscosity for each of the four concentrations given in Table 3 was plotted against ΔT, the temperature excess above the corresponding miscibility limit. These graphs are seen in Figs. 4(a) and 4(b), where the two points for negative ΔT are estimates based on the general trend of the graphs in Fig. 3(c), and the broken line graph with plotted triangles corresponds to the 70% allyl catechol mixture treated as a suspension of 30% oil in the allyl catechol.

(b) From the graphs of Fig. 4, smoothed values of the reduced viscosity were read off for $\Delta T = 0°, 10°, 20°, 30°$, etc., and plotted as functions of the concentration of allyl catechol in Fig. 5(a), where each of the plots tends to zero at 100% allyl catechol and extrapolates to a non-zero value at very small concentrations. These extrapolated values, which give the Intrinsic Viscosity, are plotted as solid circles in the lower graph of Fig. 5(c), and they show the expected steep rise as ΔT tends to zero. However, since the graphs of Fig. 5(a) have a very sharp peak in the middle, their extrapolation to zero concentration is subject to considerable error.

(c) From a theoretical stand point, it is better to reckon the mixtures with more than 50% allyl catechol as suspensions of oil in the catechol, and then to calculate the Reduced Viscosity in terms of the concentration of oil. This has been done in the last row of Table 3 for the mixture containing 70% allyl catechol. On the above basis, the graph of reduced viscosity against concentration should be symmetrical about the 50% line, and

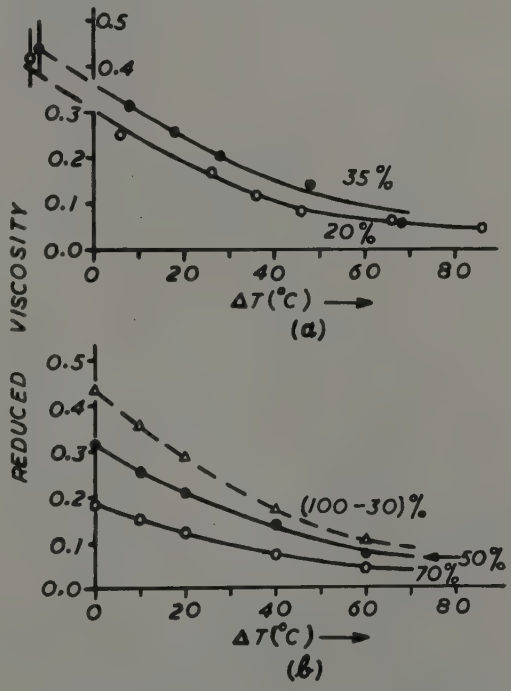

Fig. 4.—Graphs showing the Reduced Anomalous Viscosity as a function of temperature excess, ΔT, above the correspondng miscibility temperature. Reduced Viscosity = $(\Delta\nu/\nu) \div$ Concentration of allyl catechol, the concentrations being indicated alongside the respective curves. The broken-line curve is for 70% catechol treated as a 30% solution of mineral oil in allyl catechol.

this serves as an additional criterion for drawing the graphs through the points plotted in Fig. 5(b), where the mean of the values for 50% and(100-30)% allyl catechol has been plotted in order to get an accuracy comparable with the other two points. The values of Intrinsic Viscosity obtained by this

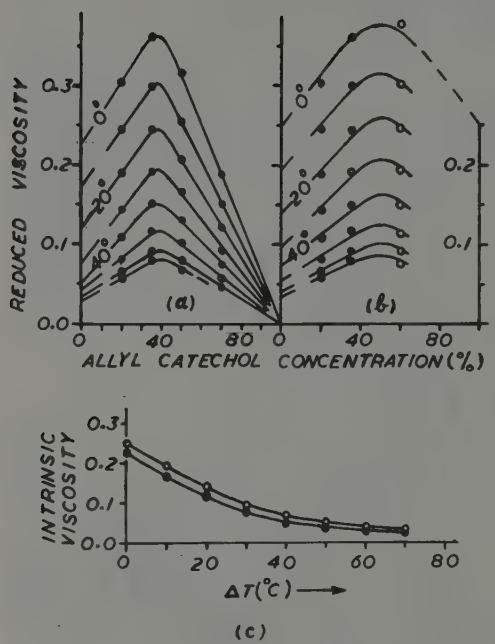

Fig. 5.—(a) & (b). Graphs for smoothed values of Reduced Viscosity for $\Delta T = 0°C.$, 10°C., 20°C., etc., as functions of allyl catechol concentration. These are used to determine the Intrinsic Viscosity as (Reduced Viscosity) conc.$\to 0$. (c).—Curves of the two differently estimated values of Intrinsic Viscosity as functions of ΔT, the temperature excess above the miscibility temperature; solid circles from Fig. 5(a), and hollow circles from Fig. 5(b).

alternative procedure are also plotted in Fig. 5(c) as hollow circles and are seen to be consistently higher than those previously obtained by about 10% to 15%.

Significance of the Temperature Dependence of the Intrinsic Viscosity

The mean of the two graphs of Fig. 5(c) can be fitted quite well by the equation.

$$\text{Intrinsic Viscosity} = 0.24 \exp.\left(-\frac{\Delta T}{30}\right), \quad (4)$$

which indicates that the seat of the phenomenon is to be found in inter-molecular forces that are still appreciable at temperatures 50°C. to 100°C. above the miscibility temperature. Visualizing the process as an incipient agglomeration of the molecules of allyl catechol near to the miscibility temperature, we may attempt to estimate the degree of this agglomeration or polymerization by means of Staudinger's empirical equation,[4] viz.,

$$P = k \times \text{Intrinsic Viscosity},$$

in which P is the degree of polymerization and k may be taken as about 300 for the type of molecules (oil and allyl catechol) that we are considering here. This makes P of the order of 3 at 100°C. above the miscibility temperature and about 70 at the miscibility temperature. The first figure of 3 is of the right order for hydrogen-bonding in solutions of allyl catechol in mineral oil, while the figure of 70 indicates the formation of quite a large aggregate at the stage when separation into two distinct phases is just about to occur. It must of course be borne in mind that these figures are to be taken as giving only the order of magnitude.

Application to Calculation of the Formula for Temperature Variation of Viscosity Depression

Since the anomalous viscosity increase analyzed above is found to persist strongly even 50°C. away from the miscibility temperatures, it should produce a significant effect on the temperature dependence of the viscosity depression previously measured with solutions of various phenolic compounds in mineral oils.[5] In order to estimate the magnitude of this effect, it is useful to study the ratio

$$R = \{(\delta\nu)_{\text{anomalous}}/(-\Delta\nu)\}$$

where $(-\Delta\nu)$ is the "normal" viscosity depression and $(\delta\nu)_{\text{anomalous}}$ is the value of the anomalous increase in viscosity measured from the "normal" curve (cf. broken line curves in Fig. 3). Values of this ratio 'R' for different temperatures are given in Table 4 for the four concentrations of allyl catechol.

Since the dependence of $\delta\nu$ and $-\Delta\nu$ on temperature is exponential, semi-logarithmic plots are given in Fig. 6 for R against ΔT, the temperature excess above the miscibility temperature. These plots are linear with nearly the same slope in every case, the mean value of the slope being 0.32 ± 0.01, whence it follows that R is given by the relation (cf. equation 4)

$$R = \frac{\delta\nu}{-\Delta\nu} = A \exp\left[-\frac{\Delta T}{62}\right] \quad (5)$$

where 'A' depends on the concentration of the allyl catechol, but shows only a small variation in the range from 30% to 70%. The exceptionally low value of 'A' for 20% allyl catechol may be attributed in part to the fact that the experimental points for concentrations upto 10% have to be used to fix the linear part of the broken line graphs

TABLE 4.—THE RATIO 'R' OF THE ANOMALOUS VISCOSITY INCREASE TO THE NORMAL VISCOSITY DEPRESSION.

Temperature (°Centigrade)		180	160	140	130	120	100	Miscibility temperature
$R=\frac{\delta\nu_{anomalous}}{-\Delta\nu}$	20% allyl catechol	0.22	0.24	0.26	0.35	0.46	0.57	94°C.
	35% ,, ,,	0.38	0.76	0.91	1.06	1.20	—	112°C.
	50% ,, ,,	0.71	1.10	1.36	1.52	1.75	—	120°C.
	70% ,, ,,	0.81	1.10	1.50	1.69	1.96	—	120°C.

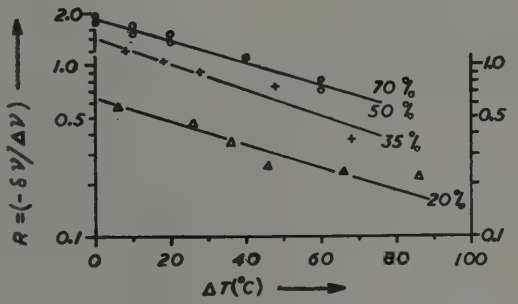

Fig. 6.—Semi-logarithmic plots showing the temperature dependence of the ratio 'R' of the anomalous viscosity increase to the "normal" viscosity depression for four different concentrations of allyl catechol from 20% to 70%.

of Fig. 3 near the origin, which will lead to a significant underestimation of the values of $\delta\nu$ in the region of 15% to 30% allyl catechol. Taking this into account, we may consider the value of 'A' as approximately constant at 1.3 ± 0.3 for mixtures containing 15% to 45% allyl catechol, which are the significant ones for determining the values of $(-\Delta\nu)_{max}$ and $(-\partial\nu/\partial x)_{x=0}$ and their variation with temperature.[5]

In an earlier communication,[1] it was shown that at a temperature of 40°C., the depression, $-\Delta\nu$, in the allyl series of compounds, (allyl benzene, allyl phenol and allyl catechol) is related to the viscosity ν by

$$\ln(-\Delta\nu) = K + 1.36 \ln \nu, \qquad (6)$$

We may consider this equation to give the ideal value, $(-\Delta\nu)_{ideal}$, of the viscosity depression in the absence of the effect of proximity to the miscibility temperature, because this effect was corrected for in allyl catechol and is negligible in allyl benzene. For examining the temperature variation in the case of allyl phenol, we now introduce the anomalous viscosity increase $\delta\nu$, which is appreciable but smaller than $(-\Delta\nu)$ at 50°C. to 100°C. above the miscibility temperature. Thus we put

$$\Delta\nu = (\Delta\nu)_{ideal} + \delta\nu = (\Delta\nu)_{ideal}\{1 - \delta\nu/(-\Delta\nu)_{ideal}\}, \qquad (7a)$$

and get

$$\ln(-\Delta\nu) = \ln(-\Delta\nu)_{ideal} - \delta\nu/(-\Delta\nu)_{ideal} = \ln(-\Delta\nu)_{ideal} - R, \qquad (7b)$$

Assuming the formula (5) for R determined above for allyl catechol to apply also in the case of allyl phenol as a first approximation, we can differentiate (7) with respect to T, to obtain

$$\frac{\partial \ln(-\Delta\nu)}{\partial T} = \frac{\partial}{\partial T}\ln(-\Delta\nu)_{ideal} + \frac{R}{62}$$

$$= \frac{\partial K}{\partial T} + 1.36\frac{\partial \ln\nu}{\partial T} + \frac{R}{62} \qquad \text{from (6)}$$

$$= \frac{\partial \ln\nu}{\partial T}\left\{1.36 + \frac{R}{62\,\partial \ln\nu/\partial T} + \frac{\partial K}{\partial T}\Big/\frac{\partial \ln\nu}{\partial T}\right\} \qquad (8)$$

Comparison with Experiments on Allyl Phenol

Examination of equation (8) shows that the quantity in curly brackets will give the value of the coefficient in an equation similar to (6) and applicable when temperature variation of $(-\Delta\nu)$ is considered for one particular compound. Since $\partial \ln\nu/\Delta T$ is of the order of $-1/50$, the effect of the second term is to decrease the value of the coefficient appreciably below 1.36, as has been experimentally observed in the case of allyl phenol and bhilawanol.[5] A quantitative estimate can be made by ignoring the third term in equation (8) and taking for $\partial \ln\nu/\partial T$ the average value, $-1/46$, obtained in the case of allyl phenol in the range of 40°C. to 85°C. This yields

$$\frac{\partial \ln(-\Delta\nu)}{\partial T} = \frac{\partial \ln\nu}{\partial T}\left\{1.36 - \frac{46}{62}R\right\} \qquad 9(a)$$

The miscibility temperatures of various concentrations of allyl phenol in the corresponding oil blend were determined experimentally to be in the region of −6°C. to −30°C., with an average of −18°C., so that the mean value of 'R' in equation 9 may be taken as $1.3 \exp\left[-\frac{62.5-(-18)}{62}\right]=0.35$ which gives

$$\frac{\partial \ln(-\Delta\nu)}{\partial T}=\frac{\partial \ln \nu}{\partial T}\left\{1.36-0.26\right\}=1.10\frac{\partial \ln \nu}{\partial T}, \quad 9(b)$$

thus indicating that, for the temperature variation of the viscosity depression in allyl phenol, an equation of the form

$$\ln(-\Delta\nu)=K^{1}+1.10\ln\nu \qquad (9c)$$

should hold. The value 1.10 calculated here for the coefficient of ln ν bears satisfactory comparison with the value 1.17 ± 0.03 determined experimentally.[5] It is believed that the remaining discrepancy can be explained by inclusion of the third term $(\partial K/\partial T)/(\partial \ln\nu/\partial T)$ of equation (8), and it is hoped to investigate this aspect further when experimental data at low temperatures is available on allyl phenol and bhilawanol.

Acknowledgements

The authors are indebted to Mr. Salim Malik for carrying out the measurements of miscibility temperatures for mixtures of allyl phenol with mineral oil.

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INFRARED ABSORPTION STUDY OF THE RELATION BETWEEN VISCOSITY AND INTERMOLECULAR HYDROGEN-BOND FORMATION FOR SOLUTIONS OF PHENOLIC COMPOUNDS IN OILS

Part II.–Solutions of Allylphenol in Mineral Oil

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(Received October 1, 1959)

The theoretical formula connecting H-bond absorbance with the relative viscosity depression, viz.

$$-\frac{\Delta\nu}{\nu} = K\frac{C}{100}\left(1-\frac{a_c/C}{a_{100}/100}\right)$$

developed in part I of this paper is now further verified with solutions of allyl phenol in mineral oil. The value of the non-dimensional constant 'K' is found to be 0.83 ± 0.06, which compares well with the value 0.77 ± 0.04 obtained for bhilawanol, $C_6H_3(OH)2C_{15}H_{27}$. This suggests that K is a generally applicable constant with a value of 0.80 ± 0.03.

Introduction

In part I of this paper,[1] some experiments were described on infrared absorption measurements of hydrogen-bond concentration in solutions of bhilawanol in mineral oil. These measurements led to the development of the following equation connecting the absorbance 'a' at the absorption peak with the viscosity depression, $-\Delta\nu$, observed in the solutions:

$$-\Delta\nu/\nu = K\left[\frac{C}{100}\left(1-\frac{a_c/C}{a_{100}/100}\right)\right] \quad (1)$$

An experimental value of 0.77 ± 0.04 was obtained for the *non-dimensional* constant K, and, in an effort to study the general applicability of equation (1), the investigations have now been extended to the case of mixtures of mineral oil and allyl phenol, which differs from bhilawanol both in the number of OH groups and the length of the side-chain.

Experimental Results

Samples of allyl phenol prepared for earlier measurements on the viscosity depression[2,3] were used in the present experiments also. The preliminary infrared curves for a few different concentrations of allyl phenol are shown in Fig. 1(a) and they indicate the desirability of using a cell thinner than 0.03 mm. for the higher concentrations. Accordingly, cells of thicknesses 0.0115 mm. and 0.080 mm. (the thicknesses being obtained from interference measurements) were used for the measurements at high resolution, which gave a lot of fine structure of the OH and H-bond peaks as shown in Fig. 1(b), the peak positions being 2.83μ (3530 cm.$^{-1}$) and 2.92μ (3420 cm.$^{-1}$), respectively. The resolution of the absorption curves into the OH and H-bond peaks (ignoring the fine structure) is shown by the thick lines in Fig. 2. The quantitative data obtained from these resolved peaks is arranged in Table 1, and the logarithmic graphs for absorbance against weight % concentration of allyl phenol are shown in Fig. 3. The slope of the graph for the OH peak is 0.96 ± 0.06 and of that for the hydrogen-bond peak is 1.42 ± 0.03. The former value is in agreement with the theoretical figure of 1.00, while the value 1.42 is in excellent agreement with the corresponding slope (1.40 ± 0.05) previously found for bhilawanol. It is to be noted here that, if molar absorbances are plotted against molar concentrations, then the scales of abcissae and

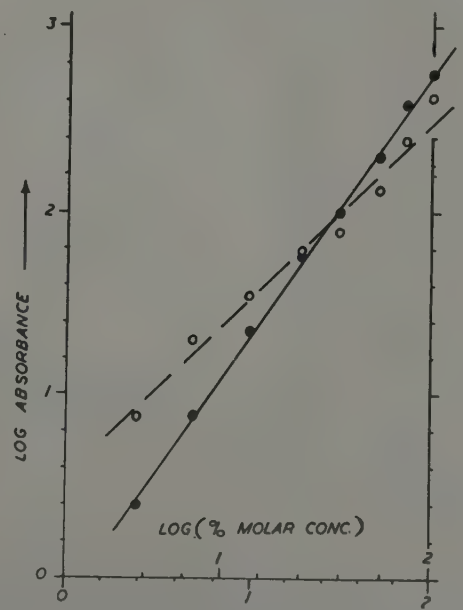

Fig. 3.—Logarithmic plots of absorbance (for 1 cm layer) against wt.% allyl phenol for the OH peak (broken line). and the H-bond peak (full line).

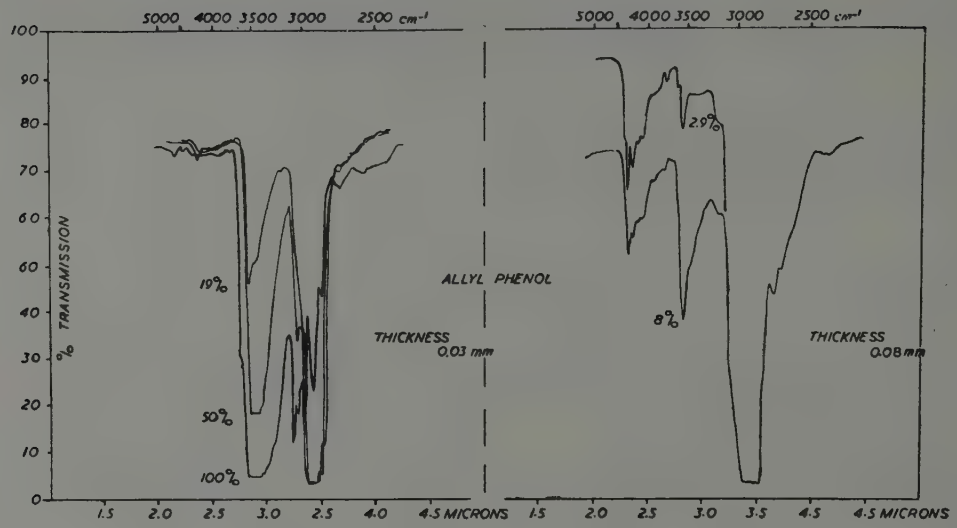

Fig. 1(a).—Preliminary infrared absorption curves for solutions of allyl phenol in H.S.D. oil blend.

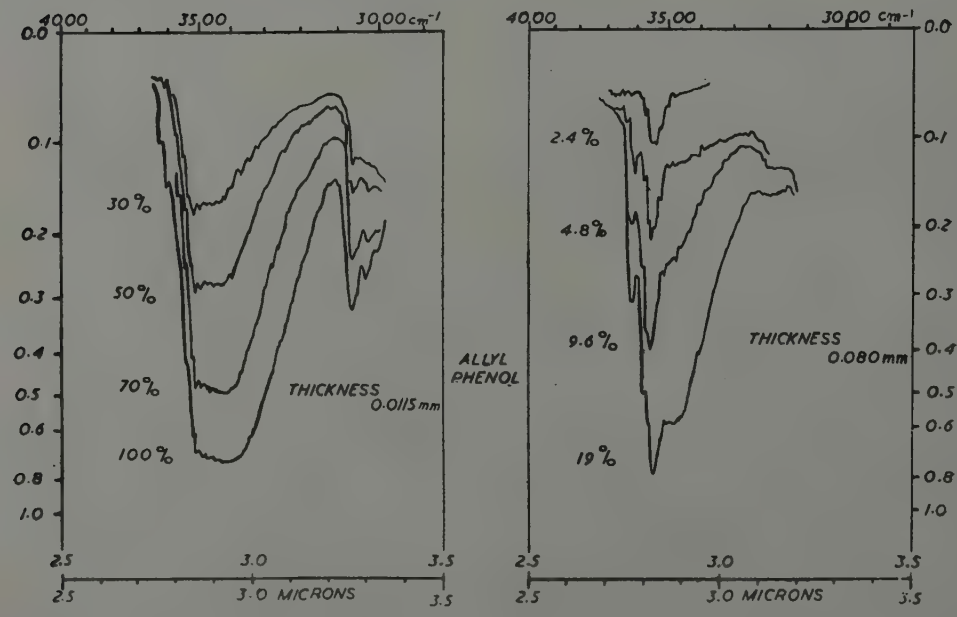

Fig. 1(b).—OH and H-bond peaks at high resolution for various concentrations of allyl phenol in the mineral oil blend.

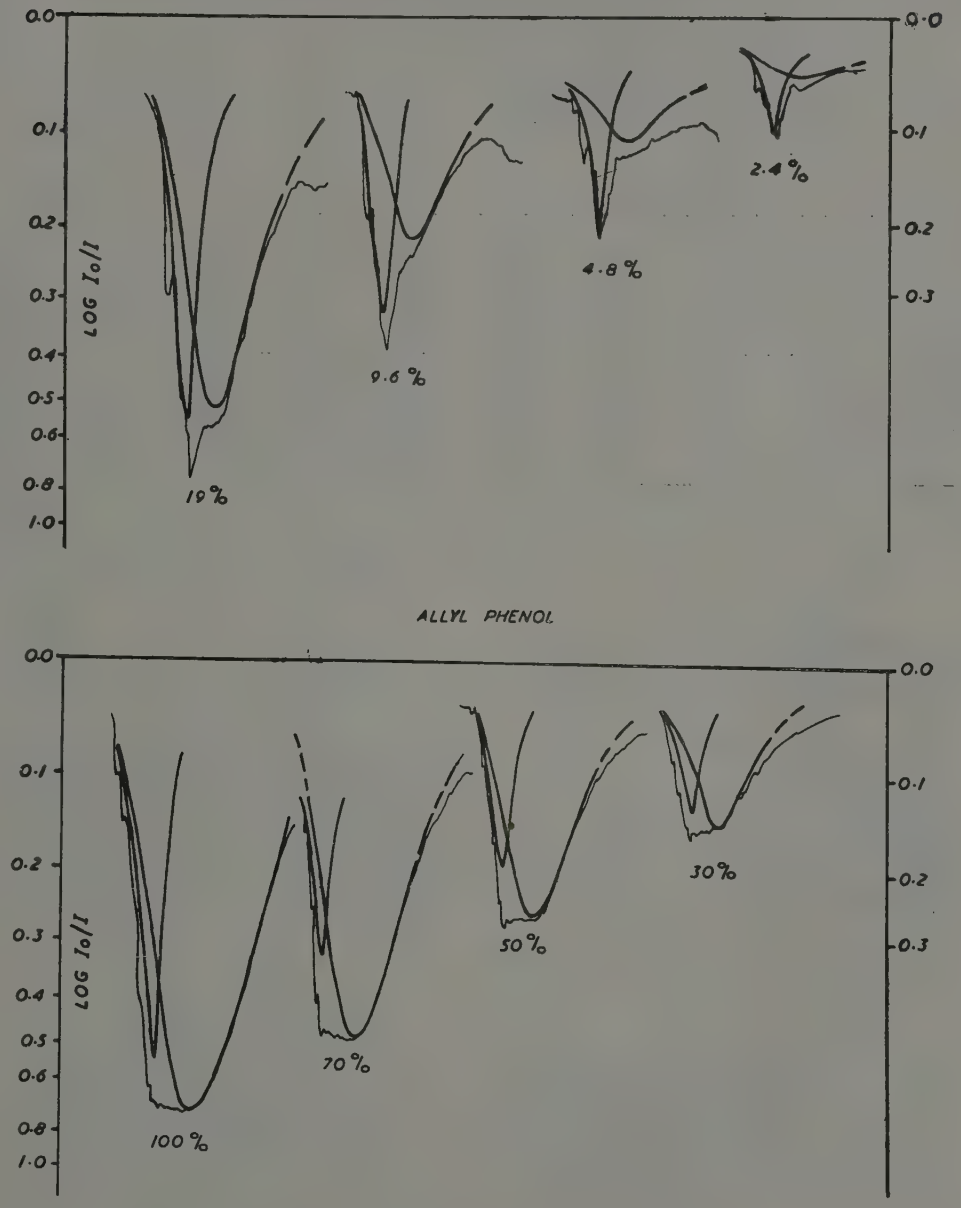

Fig. 2.—Graphical resolution of the curves of Fig. 1(b) into the component OH and H-bond absorption peaks. The ordinate scale is marked in terms of the absorbance, log (I_0/I).

TABLE I.—OH AND H-BOND PEAK HEIGHTS AND ABSORBANCES IN MIXTURES OF ALLYL PHENOL WITH MINERAL OIL.

Cell thickness mm.	% Conc. allyl phenol =C	Molar % of allyl phenol (M=134, M_{oil}=200)	Resolved peak height (log I_0/I)		a_c= Absorbance for 1 cm. layer		Logarithms		
			OH	H-bond	OH	H-bond	% Conc. =C	Absorbance OH	Absorbance H-bond
	100	100	0.48	0.64_5	417	561	2.000	2.620	2.749
	70	78	0.28	0.45	243	391	1.846	2.386	2.582
0.011_5	50	60	0.15_2	0.23	132	200	1.699	2.121	2.301
	30	39	0.09_0	0.11_5	78	100	1.477	1.893	2.000
	19.2	26	0.50	0.47	62.5	59.0	1.284	1.796	1.771
	9.6	13.6	0.28	0.18	35.0	22.5	0.983	1.544	1.353
0.080	4.8	7.0	0.16	0.06	20.0	7.5	0.681	1.301	0.876
	2.4	3.5	0.06	0.02	7.5	2.5	0.380	0.876	0.398

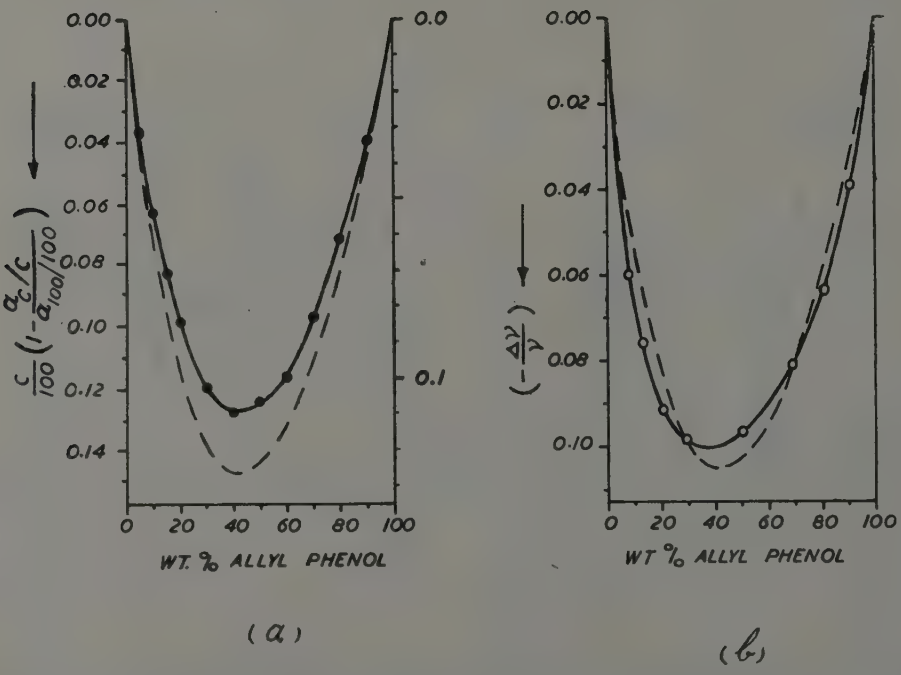

Fig. 4.—Comparison between the values of $\frac{C}{100}\left(1-\frac{a_c/C}{a_{100/100}}\right)$ calculated from infrared absorption and the graphs of $(-\Delta\nu/\nu)$ obtained from viscosity measurements on allyl phenol solutions.

ordinates are both altered in the manner indicated by the second set of markings for the abcissae in Fig. 3. This is approximately equivalent to a shortening of the graphs in both directions by a factor of nearly 0.90, and therefore leaves the slopes of the lines unaltered.

Determination of the Constant K

The values of $\frac{C}{100}\left(1-\frac{a_c/C}{a_{100}/100}\right)$ calculated from the graph for the hydrogen-bond peak in Fig. 3 are plotted in Fig. 4 alongside of the experimentally determined values of $\Delta\nu/\nu$, at the corresponding temperature[3] and give graphs similar to those for bhilawanol (Fig. 5 in part I of this paper). The graph calculated from the infrared measurements is again somewhat shallower than the experimental curve, and needs therefore to be lengthened vertically by a factor of nearly 1.16 in order to obtain good correspondence between the two curves. This adjustment gives agreement to within $\pm$ 0.08 in term of $\Delta\nu/\nu$(cf. broken curves), which is satisfactory, but is somewhat poorer than in the case of bhilawanol. The value of the constant K in equation (1) can be obtained (as before) from a comparison of the graphs of Fig. 4, and comes out to be

$$K = (0.10 \pm 0.007)/0.120 = 0.83 \pm 0.06.$$

Discussion

The value of K obtained above for allyl phenol differs by (0.06 $\pm$ 0.07) from the corresponding value (0.77 $\pm$ 0.04) for bhilawanol. The difference is thus not statistically significant, and we may therefore take the mean of the two values, namely (0.80 $\pm$ 0.03), as a *generally applicable constant* connecting the relative viscosity depression with the infrared hydrogen-bond peak absorbance by means of equation (1). Since this conclusion is based on experiments with only two different compounds, it is desirable to investigate mixtures of other hydroxylic compounds with mineral oils in order to place the above formula on a firm footing, and it is hoped to present some further results in this direction in future communications together with their theoretical implications.

It must be mentioned here that the solutions when placed in the infrared cell for absorption measurements will be heated to some extent by the passage of the infrared beam, so their temperature will be above that (37 °C.) measured in the thermostatic (beam) chamber, the difference being small, perhaps of the order of 1 °C. It can be seen that this will not produce any significant effect on the values found above for K, because the experimental curves for $\Delta\nu/\nu$ are altered by only 0.5% for a 1 °C. change in temperature.[3] Another point to be noted is that it appears from an examination of the theoretical arguments that the quantity $\Delta\nu/\nu$ in equation (1) should more precisely be $\Delta(\ln \nu)$, but the discrepancy will not be appreciable except when $\Delta\nu/\nu$ is much greater than 0.2.

Acknowledgements

The authors are indebted to Dr. Salimuzzaman Siddiqui, whose interest in these investigations has always been a source of encouragement.

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CHEMICAL AND X-RAY EXAMINATION OF AN ASBESTOS-LIKE MINERAL OCCURRING IN HINDUBAGH

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(Received October 1, 1959)

A fibrous asbestos-like mineral from Hindubagh has been examined chemically and with the help of X-ray diffraction patterns. It has been found to consist of 50% brucite ($Mg(OH)_2$), 40% dolomite ($MgCO_3CaCO_3$) and at most 10% chrysotile asbestos. The chrysotile component has been identified by means of fibre and powder X-ray patterns of the residue obtained from acid-treatment of the original sample.

A sample of fibrous material, reportedly asdestos, collected from Hindubagh, was received through the courtesy of the Geological Survey of Pakistan for a study of its possible uses in the asbestos industry. The bulk of the sample consisted of well-formed long fibres in rock formations. The fibres were greenish-white, silky smooth and lusturous, their texture varying from soft to brittle hard (the brittle hard material being predominant). Because various varieties of asbestos, each suited for specific uses, are found to occur in nature, it was considered essential, in the first instance, to identify and classify the mineral.

Axial-length Measurements along Fibre-axis

In order to apply H.V. Anderson and G.L. Clark's X-ray method[1] for the classification of asbestos (based on axial-length measurements along fibre axis and the weight lost by variously treated samples), X-ray patterns of hand-picked fibres were taken with a Cylindrical Bernal camera, using filtered Cu K radiation. The patterns thus obtained are reproduced in Figs. 1(a) and 1(b), Fig. 1(a) being the pattern obtained with the untreated fibres, while Fig. 1(b) shows the pattern of the fibres after heating at 1000°C. for 2 hours. Both these patterns show sharp powder lines, super-imposed upon which are several strong and fairly sharp diffraction spots arranged on layer lines, indicative of a good fibre structure oriented around the axis. In Table 1, the values of the axial lengths along the fibre axis, as calculated from these two patterns and the weights lost with standard chemical treatments are compared with the nearest values from the standard data given by Anderson et al.,[1] picrolite showing the highest loss on treatment, and chrysotile having the next highest in the list.

Examination of the data of Table 1 suggests that the mineral under study is essentially different from any of the listed varieties of asbestos. This was confirmed by the X-ray pattern taken after leaching with 1:1 hydrochloric acid for 12 hours.

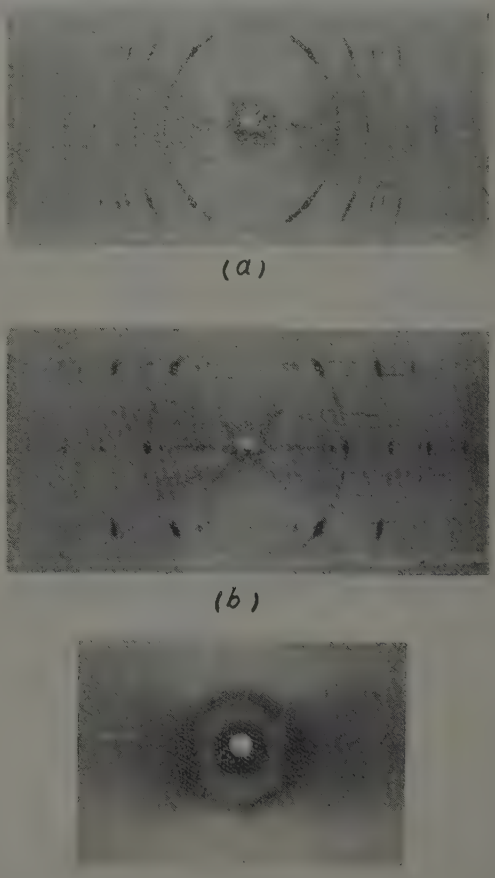

Fig. 1.—X-ray fibre patterns of the asbestos-like mineral taken with a normal Bernal camera, using filtered Cu K radiation: (a) untreated fibres, (b) fibres after heating at 1000°C for 2 hours, and (c) fragment from residue after leaching with 1:1 HCl for 12 hours.

TABLE 1.—AXIAL LENGTHS ALONG FIBRE AXIS OF THE UNTREATED AND TREATED SAMPLES, TOGETHER WITH WEIGHT LOSSES: COMPARED WITH SOME STANDARD DATA.

Treatment given	Weight percent loss on treatment			Identity period (axial length) along fibre axis		
	Picrolite	Unknown	Chrysotile	Picrolite	Unknown	Chrysotile
1. Untreated sample	—	—	—	~ 5 A	3.14A	5.06A±0.14
2. Heat-treated sample at 1000°C. for 2 hrs.	35.1	35%	17.3±6.0	—	2.96A	—
3. Acid-treated sample (leached in 1:1 HCl for 12 hours)	85.9	95%	55.6±2.7	—	—	—

While the bulk of the material dissolved in the acid solution, some fragments of the residue gave the very poor pattern shown in Fig. 1(c), which is conspicuously different from the first two and has four faint spots, yielding an axial length of 4.8 ± 0.2A, which would agree with that of chrysotile. This suggests that, whereas the bulk of the material is not identifiable as asbestos, it probably contains a small fraction (about 10%) of some form of asbestos. A more detailed chemical and X-ray analysis was therefore undertaken to identify these and other possible components of the sample.

Chemical Analysis

A representative fraction of about 5 lbs. was taken from the bulk sample and crushed in a jaw crusher. By repeated crushing and sampling (by the quartering method), a finely-ground representative sample was obtained. This was oven-dried at 110°C. and then chemically analysed. The mean results of half a dozen analyses are given in Table 2. The deficit of 3·3% can be accounted for by alkali metals, etc.

TABLE 2.—CHEMICAL ANALYSIS OF THE UNTREATED SAMPLE

MgO	41.1%	Fe_2O_3	3.8%
CaO	10.9%	Loss on ignition	36.7%
SiO_2	4.2%	Total	96.7%

Generally the different varieties of commercial asbestos fibres vary in their SiO_2 content[2] from 37-62%, and thus the significantly low SiO_2 content of the sample would at best account for about 10% asbestos. Further, magnesium and calcium are present in appreciable quantities, and the loss on ignition is quite high, and would correspond to the presence of a carbonate or a hydrate in appreciable quantities. The carbonate radical was detected by hydrochloric acid treatment, but its quantitative estimate was not carried through. Thus magnesium carbonate or hydroxide may be the major phase present in the sample.

X-ray Powder Patterns

For a complete identification of the actual component phases, recourse was again taken to X-ray diffraction methods. One of the phases can be identified as brucite, $Mg(OH)_2$, from the powder lines on the fibre pattern of the untreated sample (Fig. 1(a)). Furthermore, the fibre pattern of the heat-treated sample (Fig. 1(b)) also gave sharp powder lines, which correspond to MgO. A powder photograph of the representative sample was next taken with filtered Cu K radiation in a 9-cm. diameter powder camera. This photograph is reproduced in Fig. 2, along with comparison patterns of brucite, of asbestos, and of a 1:2 hydrochloric acid treated sample. In Table 3 are given the d-values of the lines of the experimental pattern together with those of comparison standards.

After accounting for the brucite lines on the experimental pattern, some prominent lines remain outstanding, those with d-values of 2.89, 2.19,

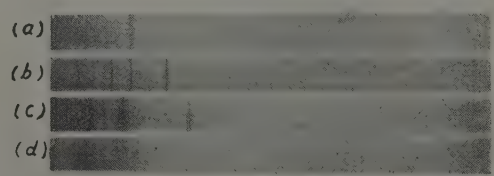

Fig. 2.—X-ray powder patterns of the mineral and of comparison standards, taken with 90 mm. dia. camera using filtered Cu K radiation: (a) standard sample of Brucite ($Mg(OH)_2$), (b) untreated mineral, (c) Chrysotile asbestos, and (d) residue from acid treatment of mineral.

2.01A (all visible in Fig. 2) being quite strong. A search in the A. S. T. M. card index[3] showed that they could correspond with the strong lines of dolomite ($MgCO_3.CaCO_3$), the complete powder data of which was found to account for most of the outstanding lines (cf. Table 3). A semi-quantitative estimate from the intensities of the powder lines indicates a ratio of 5:4 for brucite and dolomite. Allowing 10% for any other phases (asbestos, etc.), this gives 50% brucite and 40% dolomite, which leads to an estimate of 40% for magnesium oxide and 12% for calcium oxide, in good agreement with the chemical data of Table 2.

TABLE 3.—COMPARISON OF THE POWDER PATTERNS OF THE UNTREATED AND ACID-TREATED FIBROUS MINERAL WITH THOSE OF VARIOUS STANDARDS.

Untreated sample	Std. data of $Mg(OH)_2$ (brucite)	Std. data of $Mg.CO_3$. $CaCO_3$ (dolomite)	Std. data of chrysotile	Sample treated with HCl
—	—	—	8.0 (40)	7.94 W
7.52 W	—	—	7.3 (90)	7.10 M
4.80 S	4.76(60)	—	4.93(10)	—
—	—	—	4.46(60)	4.43 M
3.67 WF	—	3.71(2)	3.64(100)	3.63 W
—	—	—	—	3.37 F
2.89 S	—	2.89(100)	—	—
2.66 W	—	2.68(2)	—	—
2.52 W	—	2.55(2)	—	—
—	—	—	2.44(70)	2.49 M
2.36 S	2.37(100)	—	—	—
—	—	2.40(13)		
2.19 M	—	2.19(40)		
2.05 F	—	2.06(2)		
2.01 M	—	2.02(20)		
1.84 F	—	1.85(2)		
1.79 S	1.79(80)	1.80(40)		
1.57 M	1.57(70)	1.57(2)		
1.54 W	—	1.55(10)	1.52(90)	1.54 M
1.49 WM	1.49(60)	1.50(1)		
1.46 F	—	1.47(6)		
1.43 F	—	1.42(1)		
1.38 F	—	1.39(8)		
1.37 M	1.37(60)	—		
1.33 F	—	1.34(2)		
1.30 W	1.31(60)	1.30(1)		
1.26 F	—	1.27(1)		
1.23 F	—	1.24(2)		
1.20 F	—	1.20(1)		
1.18 F	1.18(50)	1.17(1)		
1.11 F	—			
1.10 WF	—			
1.09 WF	1.09(20)			
1.03 W	1.03(40)			
1.00 W	1.00(60)			

It may here be mentioned that the presence of magnesite ($Mg\ CO_3$) bands in the asbestos samples have been reported by investigators in the Geological Survey of Pakistan. Therefore, the powder data of the pattern of magnesite and its hydrates, as given in the A.S.T.M. card index, was also looked up but they were not found to fit the experimental pattern at all.

Brucite and dolomite account for nearly all the lines of the pattern, and only a few weak and faint lines remained unexplained. Amongst these outstanding lines, a line at d=7.5A and the other at d=3.67A were quite significant; they correspond with the strongest lines of chrysotile, already identified in the fibre pattern of Fig. 1(c), and the complete data of whose pattern also agreed well with the remaining powder lines. This phase, however, must be present only in small quantities, and so in order to confirm its presence in the original sample, attempts were made to separate it from the bulk material.

The Chrysotile Component

Hydrochloric acid (1:2) successfully separated out the chrysotile component as a residue (weighing about 6% of the total weight of material treated by the acid) without disturbing the lattice of this phase. The powder pattern of this sample, also reproduced in Fig. 2, is seen to be very diffuse. Nevertheless, it compares well with the standard asbestos (chrysotile) pattern (Table 3).

The mean of three separate analyses of this acid-treated sample are given in Table 4. The deficit of 2.2% can be accounted for by alkali metals etc.

The rather high value of SiO_2, which should be about 44% for chemically pure chrysotile, is attributable to free silica present along with the extracted fibres. The figure for magnesium is a little low, perhaps due to attack of 1:2

TABLE 4.—ANALYSIS OF RESIDUE FROM ACID-TREATMENT.

SiO_2	51.3%	MgO	25.4%
Fe_2O_3	1.1%	Combined water and CO_2 (traces)	17.7%
Al_2O_3	0.9%		
CaO	1.4%	Total	97.8%

hydrochloric acid, pure chrysotile containing 35% (approximately) magnesia. Thus the chemical analyses lend some support to the conclusion that the acid treated residue is chrysotile.

Conclusion

From the foregoing study, it is concluded that brucite and dolomite represent the major phases in the sample, while chrysotile (asbestos) forms only a small fraction (<10%) of the total weight of the material. Thus these three phases complete the list of the constituents of the sample.

Regarding the origin of the brucite (magnesium hydroxide), it may be conjectured that it is formed as a decomposition product of asbestos, as noted by earlier workers[4]—"The mineral is found with magnesite and dolomite in serpentine as a result of the decomposition of magnesium silicates".

Acknowledgements

The authors are grateful to Mr. A.H. Chotani and Dr. M.M. Qurashi for their valuable suggestions, and to Mr. M. Latif Ahmed for help in some of the X-ray work.

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STRUCTURAL STUDIES ON THE MAGNETIC FRACTION OF STEAM-TREATED MAKERWAL COAL

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(Received October 1, 1959)

In the preliminary study, both sulphur and oxygen were found to be significant elements of the magnetic fraction obtained from Makerwal coal, steam-treated at a temperature of 350°C., and the available data showed them to be present as (Fe_3O_4+S). The present investigation attempts to elucidate precisely the nature and quantities of the phases containing this sulphur. Measurements with a Guoy magnetic balance at a series of different field strengths show a rapid increase in both para and ferro-magnetic properties of the treated coal when the treatment is carried out at 300°C., or above. A comparison of these measurements with chemical estimations of total iron, total sulphur and pyritic sulphur indicates that at 400°C. only a fifth of the iron is converted into the ferromagnetic Fe_3O_4, and provides evidence for the existence of the highly paramagnetic Fe_nS_{n+1}, which is apparently produced as an intermediate product in the conversion of FeS_2 into Fe_3O_4. Successive magnetic separations carried out in the dry state gave three strongly magnetic fractions, but failed to provide any appreciable separation between the para and ferro-magnetic components.

An X-ray powder diffraction analysis of these magnetic fractions and of the untreated and treated coal as such shows that, whereas the untreated coal contains kaolinite and FeS_2 as the major crystalline phases (amounting to 5% and 2% respectively), the first magnetic fraction contains four major iron-bearing phases, viz., Fe_nS_{n+1}, FeS2, FeS and Fe_3O_4, in the approximate percentages, 5, 5, 2 and 4, respectively. These figures lead to an estimate of 9% iron and 6% sulphur, in fair agreement with the chemically determined values of 8.8% and 7.2%. The ratios of these four constituents are essentially the same in the treated coal and the three successive magnetic fractions.

The anomalous preponderance of Fe_3O_4 and the near absence of iron sulphides noted in the earlier communication are corroborated by a better X-ray pattern of the old concentrated sample, and are probably due to preferential concentration of the Fe_3O_4 in the wet magnetic concentration process that was used in the previous work.

Introduction

Preliminary work on the magnetic fraction obtained from the residue remaining behind after high temperature steam-treatment of Makerwal coal had shown[1] that, although the pyritic material appears to have been converted by steam-treatment into Fe_3O_4 (with a possibility of formation of Fe_nS_{n+1} up to the extent of 5%), the magnetic fraction still carries as much as 8% sulphur. In order to elucidate the status of this sulphur in the magentic fraction, and to study further the reactions involved in the steam-treatment, it was decided to undertake magentic measurements and detailed X-ray investigation on the untreated coal and on samples of coal treated with superheated steam at different temperatures upto 400°C. The results of these studies are presented in this communication and are correlated with the chemical analysis of the various treated and untreated samples.

1. Magnetic Measurements and Chemical Analysis

Magnetic susceptibilites (χ) of untreated Makerwal coal and of the different samples of the steam-treated coal were determined with a Gouy magnetic balance at different field strengths ranging from 3,000 to 5,200 oersteds. This balance, which had been constructed in this laboratory, was first standardised approximately with a search coil and ballistic galvanometer, and then more accurately with $CuSO_4 \cdot 5H_2O$ and $NiSO_4 \cdot 7H_2O$, and the final readings were found to be reproducible to an accuracy of 1%. The results of magnetic measurements on two samples of untreated Makerwal coal and on samples of differently treated Makerwal coal are plotted in Figs. 1(a) to 1(e) with $\chi \times 10^6$ as ordinates and 10,000/H as abscissae, and satisfactory straight lines can be drawn through these points.

The susceptibilities (χ_p) of the paramagnetic part and Saturation Magnetisation (σ_f) corresponding to the ferromagnetic part of each sample were read off from the ordinate intercepts and the slopes of the graphs in Fig. 1, and the means of two determinations, each with a new sample, are given in Table 1. A 50 to 80 fold increase in values after steam-treatment at the temperature of 400°C. is evident, cf. also Fig. 2(a).

TABLE 1.—MAGENTIC DATA FOR TREATED AND UNTREATED COAL SAMPLES.

Description of the sample	$\chi_p \times 10^6$	$\sigma_f \times 10^2$	$\frac{\sigma_p}{\chi_f} \times 10-4$
(A) Untreated Makerwal coal	1.5±0.2	0.76±0.05	0.54
(B) Makerwal coal steam-treated at 280°C. for 4 hrs.	3.2±0.3	1.10±0.1	0.35
(C) Makerwal coal steam-treated at 300°C. for 10 hrs.	16.2±1	9.7±0.5	0.60
(D) Makerwal coal steam-treated at 350°C. for 10 hrs.	56±3	48±2	0.86
(E) Makerwal coal steam-treated at 400°C. for 10 hrs.	77±3	61±3	0.79

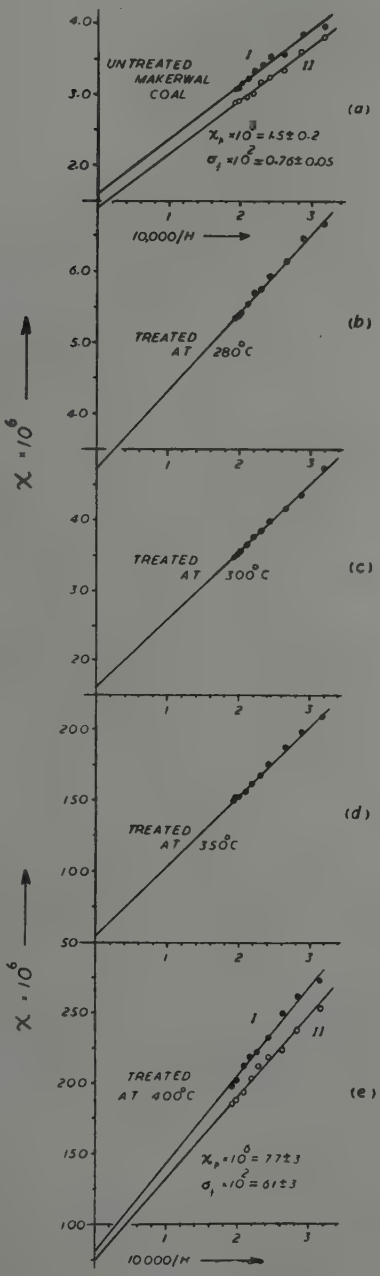

Fig. 1(a)-(e).— Plot of the magnetic susceptibility of untreated and variously treated samples of Makerwal coal measured at several different field strengths. The intercept at 1/H=o gives the paramagnetic susceptibility χ_p. The variation between the two samples in Fig. 1(a) and Fig. 1(e) is mostly attributable to sampling errors.

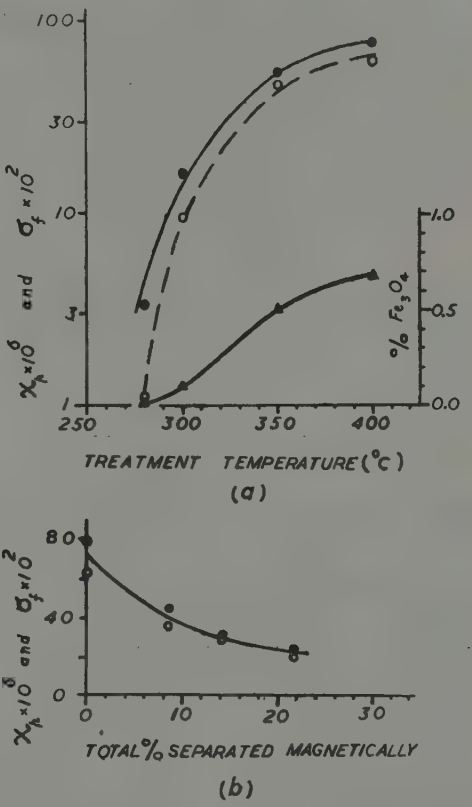

Fig. 2.—(a) Curves showing the dependence on treatment temperature of the paramagnetic susceptibility χ_p (solid circles) and the ferromagnetic saturation magnetization σ_f (hollow circles), both plotted semi-logarithmically. The triangles show the variation of % Fe_3O_4 as determined from the magnetic measurements.

(b) χ_p and σ_f plotted for the residues from successive dry magnetic separations, showing the initial rapid fall in both quantities.

On the basis of the earlier X-ray and chemical work (in spite of the possible presence of some paramagnetic Fe_nS_{n+1}), the principal ferromagnetic constituent of the above samples is Fe_3O_4, and its percentage can, therefore, be estimated fairly well on the basis of the standard value of σ_f for Fe_3O_4, viz., $93.3 \times (1 - 19/H)$ at 19°C. as per International Critical Tables.[2] These estimates are shown in column 5 of Table 2, alongside of data from chemical analyses for iron and sulphur, which show that, even in the coal treated at 400°C., the iron in the form of Fe_3O_4 constitutes only one fifth of the total iron found by chemical analysis. The rest of the iron may therefore be expected to

be present in the form of other simple inorganic compounds, probably sulphides and oxides.

TABLE 2.—CHEMICAL DATA FOR TREATED AND UNTREATED COAL SAMPLES.

Description of sample	% Total iron determined volumetrically	% Total sulphur	% Pyritic sulphur, mainly as $FeS_2+FeS+Fe_nS_{n+1}$	% Fe_3O_4 from magnetic measurements
(A) Untreated Makerwal coal	2.09	4.6	1.2	<0.01
(B) Makerwal coal steam-treated at 280°C. for 4 hours	2.11	4.4	—	0.01
(C) Makerwal coal steam-treated at 300°C. for 10 hours	2.20	3.8	—	0.10
(D) Makerwal coal steam-treated at 350°C. for 10 hours	2.65	3.3	1.7	0.51
(E) Makerwal coal steam-treated at 400°C. for 10 hours	2.75	3.1	1.8	0.68

Some sulphate → sulphide
Some sulphide → oxide

2. Evidence for Fe_nS_{n+1}

It is seen from Table 2 and Fig. 2(a) that the formation of Fe_3O_4 in appreciable quantities does not begin until steam at a temperature of 300°C. is used, and that it reaches nearly its maximum value at 400°C. The ratio $\frac{\sigma_f}{\chi_p} \times 10^{-4}$ in the last column of Table 1 first shows a decrease (for the steam-treatment at 280°C.). This is replaced by a rapid increase for treatment at temperatures of 300°C. and above. This indicates a relatively higher production of paramagnetic material in the treatments at lower temperatures and of ferromagnetic material at higher temperatures, which would suggest that the paramagnetic material is *an intermediate stage* in the formation of the ferromagnetic component, Fe_3O_4. Also it appears that, after allowing for nearly 20% loss of volatile matter, there is actually a small increase in the pyritic sulphur, which can probably be attributed to the breakdown of the sulphates, which are present to the extent of about 0.5%.

It may be deduced that, during the first stage of steam-treatment, the chemical changes in the iron-bearing phases are presumably the conversion of the sulphates and higher sulphides into lower sulphides and oxides other than Fe_3O_4, viz.,

$$FeS_2 \rightarrow FeS + S \text{ and } FeS + HOH \rightarrow FeO + H_2S,$$

$$\text{and } 2FeO + HOH \rightarrow Fe_2O_3 + H_2.$$

Of these products, Fe_2O_3 and FeS are both paramagnetic. The very high values of χ_p in Table 1 rule out the identification of this intermediate paramagnetic phase as α-Fe_2O_3, and a sulphur compound is thus indicated. Megneto-chemical studies of Haraldsen[3] and others have shown that compounds in the iron-sulphur system with sulphur in the range of 52.5 to 55 atom% have very high magnetic susceptibilities, of the order of 1100 to 1300×10^{-6} at 20°C. at infinite field strength. Thus, on the basis of the above data, it would be reasonable to presume the existence in the steam-treated samples of *several percent* of a compound of some composition within this range, which would correspond to Fe_nS_{n+1} with values of 'n' between 5 and 10.

3. Magnetic Separation in the Dry State

Attempts were next made to effect a magnetic separation of the paramagnetic and the ferromagnetic phases present in the coal treated at 400°C. A small horse-shoe magnet of field strength $H \simeq 500$ gauss near the poles was employed for this purpose. To avoid the possibility of distrubance of water-soluble material or of any mechanical or other interaction with water, dry separation was used in the first instance. The magnet was brought in contact with the powdered steam-treated sample and the particles adhering to the magnet were tapped of into another container. This process, repeated 20 times, enabled 8.6% of the sample to be separated. This was labelled 'First Magnetic Fraction' and the residue was named 'First Magnetic Residue'. In like manner, the second and third magnetic fractions were separated, and the magnetic susceptibilities of the successive magnetic fractions as well as of the residues were measured. Because the susceptibilities of the magnetic fractions are exceedingly high, they were determined by dilution with untreated coal. The results are shown in Table 3.

Deviations from additivity relationship in the susceptibilities of the magnetic fractions and the residues are largely accounted for by the fact that loss of even a trace of the highly magnetic material or a slight error in its weighing would magnify the error in the calculated susceptibility.

$\chi_p \times 10^6$ and $\sigma_f \times 10^2$ for the three residues after magnetic suparation are plotted in Fig. 2(b),

TABLE 3.—MAGNETIC DATA FOR SUCCESSIVE MAGNETIC SEPARATIONS.

Order of magnetic separation	%Magnetically removed	$\chi_p \times 10^6$		$\sigma_f \times 10^2$		$(\sigma_f/\chi_p) \times 10^{-4}$	
		Residue	Separated fraction	Residue	Separated fraction	Residue	Separated fraction
Unseparated sample	0.0	77±3	—	61±3	—	0.79±0.05	—
First fraction	8.6	45±2	239±9	36±2	210±9	0.80±0.06	0.88±0.06
Second fraction	8.6+5.5=14.1	31±2	176±7	30±1	160±7	0.97±0.06	0.90±0.06
Third fraction	14.1+7.5=21.6	24±2	94±4	20±2	78±4	0.83±0.06	0.84±0.06
					Mean:	0.87±0.05	0.87±0.05

and the mean curve through these points shows the progressive decrease of the magnetic material in the residue with each magnetic separation, and indicates that this method of separation would not be of any practical utility after about 15% of the material is separated as the magnetic fraction. The ratio $(\sigma_f/\chi_p) \times 10^{-4}$ in all three magnetic fractions is not very different from that in the unseparated material, indicating that no appreciable preferential separation of the ferromagnetic component has been obtained. However, the separated magnetic fractions could be used for X-ray diffraction and chemical analyses.

4. X-ray Diffraction Analysis

In order to confirm and amplify the foregoing results and to obtain detailed information about the phases, X-ray powder patterns of the untreated coal, the steam-treated coal and the different magnetic fractions (separated as above) were taken on a Seifert 11.4 cm. camera using filtered chromium K-radiation and exposure times of 15 to 20 hours. Complex patterns showing the presence of many phases were obtained, as against the one major phase (i.e. Fe_3O_4) reported in the preliminary investigation.[1] The patterns of kaolinite, of the treated and untreated coal and of the first magnetic fraction are shown in Fig. 3(a) (top), while Fig. 3(b) (below) shows those of the third magnetic fraction and the constituent phases for comparison. The measured d-values of the lines are recorded in Table 4, together with the standard data for the relevant phases. As many as five phases can be definitely identified in the magnetic fractions of the steam-treated sample, viz., Fe_3O_4, FeS_2, Fe_nS_{n+1}, FeS and kaolinite, with a possibility of traces of α-Fe_2O_3.

(a) UNTREATED COAL

The powder pattern of the untreated coal is in general very poor, but it shows four strong lines corresponding to d-values of 3.53A, 2.70A, 1.63A and 1.485A. The A.S.T.M. Card Index data[4] shows that the strong lines at 2.70A and 1.63A correspond with the strongest lines of FeS_2, the complete data for which can be accounted for on the experimental pattern (cf. Table 4). The other strong lines of d-values 3.53A and 1.485A correspond to the strong lines of kaolinite, the data of which agrees with most of the outstanding lines. The line of d-value 7.15A, which is one of the strongest lines of kaolinite could not be observed on the experimental pattern because of the very dark back-ground in this low-angle region of the film. However, this line could be easily identified in the later patterns of the magnetic fractions of the steam-treated coal samples, which have a much lighter back-ground in that region.

FeS_2 and kaolinite thus appear to be the major crystalline phases of the untreated sample, their rough percentages being estimated as 5 and 2, respectively, from the intensities of their characterstic lines. After accounting for the lines attributable to these two major phases, a few faint lines remain outstanding; two of these, at 1.83A and 1.69A, can be identified with the distinctive lines of α-Fe_2O_3, the other important lines of which are also observable on the pattern. Thus α-Fe_2O_3 also seems to be present, but in very small quantities (<0.5%). Traces of SiO_2 and Fe_3O_4 are also indicated by their respective strongest line.

(b) STEAM TREATED COAL AND ITS MAGNETIC FRACTIONS

The pattern of the coal after steam treatment at

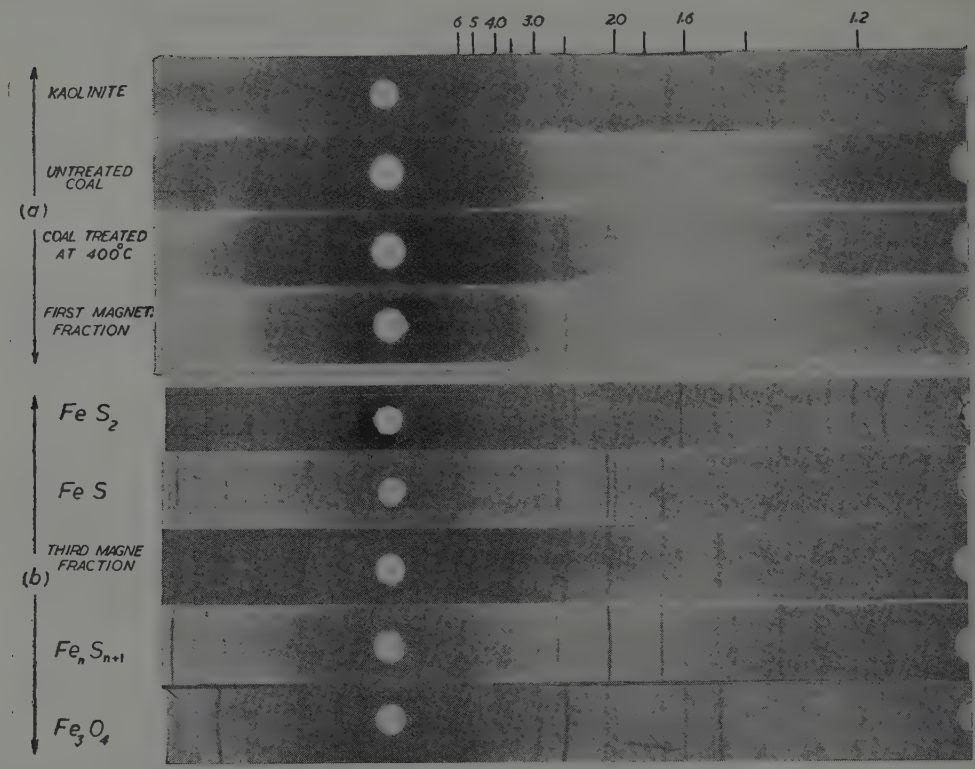

Fig. 3.—(a) X-ray diffraction patterns of untreated Makerwal coal, the steam-treated coal and the first magnetic fraction, with a comparison pattern of kaolinite at the top. Patterns taken with a 114 mm. dia. camera, using filtered Cr K radiation; the scale at the top indicates approximately the d-values in Angstroms.

(b). X-ray diffraction pattern of the third magnetic fraction alongside those of the iron sulphides and oxide that form its constituent phases. The sample of FeS contains a considerable quantity of free iron, some of whose lines are clearly visible, viz., at 2.03A. and 1.17A.

400°C. shows the presence of almost all the lines of the untreated coal pattern, although with considerable intensity differences, and further shows the emergence of several prominent new lines corresponding to d-values of 3.00A, 2.63A, 2.06A, 1.72A and 1.475A, suggesting the formation of several new phases as a result of steam-treatment. The strong magnetic character of the sample indicates the formation of some iron-bearing phase of high magnetic susceptibility, and therefore, an attempt was made to identify the new phases with the standard data of the more probable compounds, namely FeS_2, Fe_nS_{n+1}, FeS, Fe_3O_4, FeO, and α-Fe_2O_3, as given in the A.S.T.M. card index[4] and also by reference to the work of Haraldsen and others.[3] Whereas the powder patterns of FeO, Fe_3O_4 and α-Fe_2O_3 are quite distinct, it appears from the data of FeS and Fe_nS_{n+1} that their patterns are not readily differentiated (cf. Table 4). Thus the strongest lines of FeS are seen to be those at 2.09±0.01A (Intensity 100) and 2.66± .02A (Intensity 80), while those of Fe_nS_{n+1} are at 2.06A (Intensity 100) and 2.63A (Intensity 50).

For the above reasons, for the proper identification of all the phases produced, it was considered expedient to use the patterns of the magnetic fractions separated from the treated coal because they give very much better powder patterns than the whole coal sample. As seen in Figs. 3(a), 3(b) and 4, the patterns of the three successive magnetic fractions are essentially the same, in agreement with the indications of the magnetic measurements.

(*i*) *Sulphides of Iron.*—The strongest lines of the

TABLE 4.—COMPARISON OF THE d-VALUES (IN ANGSTROMS) OF THE LINES OBSERVED ON THE X-RAY DIFFRACTION PATTERNS OF THE TREATED AND UNTREATED COAL AND THE MAGNETIC FRACTION WITH THE STANDARD PATTERNS OF THE IRON SULPHIDES AND OXIDES.

Untreated coal	Steam-treated coal	Kaolinite	1st magnetic fraction of coal treated at 400°C.	Minus kaolinite lines	FeS_2	FeS* Troilite	Fe_nS_n+1	Fe_3O_4 and γ-Fe_2O_3	α-Fe_2O_3	FeO
Very heavy background in this region	Very heavy background in this region	7.15(100) 4.45(50) 4.35(60) 4.17(60) 4.12(30)	7.0 (70) 4.4 (20) — 4.15(30) —	— — — — —	— — — — —	— 4.72(20) — — —	— — — — —	— — — — —	— — — — —	— — — — —
— — 3.53 (M) — 3.33 (F)	(S) 3.8 — (S) 3.55 — —	3.84(40) 3.73(20) 3.57(100) — 3.37(40)	3.85(15) — 3.53(60) 3.50(10) 3.33(10)	— — — 3.50(10)** —	— — — — —	3.82(20) — — — —	— — — — —	— — — — —	— 3.68(70) — — —	— — — — —
— — 3.10 (F) — 2.80 (VF)	— — — (S) 3.00 —	— 3.14(20) 3.09(20) — —	3.24(10) — 3.10(20) 2.99(80) —	3.24(10)** — — 3.00(80) —	— — 3.128(36) — —	— — — 2.98(80) —	— — — 2.97(10) —	— — — 2.97(28/34) —	— — — — —	— — — — —
— 2.70 (M) — 2.54 (W) —	— (W) 2.72 (W) 2.63 (D) 2.56 —	2.75(20) — — 2.55(70) 2.52(40)	2.76(20) 2.70(60) 2.64(30) 2.55(30) 2.51(100)	— 2.70(60) 2.64(30) — 2.51(80)	— 2.709(84) — — —	— 2.68(90) — 2.54(20) —	— — 2.63(50) — —	2.78(-/19) — — — 2.53(100)	— 2.69(100) — — 2.51(80)	— — — — —
2.47 (F) 2.42 (W) 2.36 (F) 2.32 (WM) 2.27 (W)	(D) 2.49 (W) 2.41 — (W) 2.33 —	2.486(80) — 2.374(70) 2.331(90) 2.284(80)	2.49(30) 2.40(80) — 2.33(70) 2.29(30)	— 2.40(80) — 2.33(25) —	— 2.423(66) — — —	— — — 2.32(70) —	2.45(5) — — — 2.26(5)	— 2.42(11/-) — — —	— — — — —	2.486(80) — — — —
2.20 (W) — — — —	(W) 2.21 — — — (MS)2.06	2.182(30) — 2.127(20) — —	2.20(60) — — 2.095(40) 2.06(100)	2.20(45) — — 2.095(40) 2.06(100)	2.218(52) — — — —	— — — 2.10(100) —	— — — — 2.06(100)	— — — 2.10(32/24) —	2.20(70) — — — 2.07(10)	— 2.153(100) — — —
1.99 (W) — 1.90 (W) — 1.83 (F)	— — (W) 1.915 — —	1.985(70) 1.935(40) 1.892(20) 1.865(5) 1.825(40)	1.98(30) — 1.90(60) 1.88(10) —	— — 1.90(50) 1.88(8) —	— — 1.915(40) — —	— — 1.90(20) — —	— — — 1.88(5) —	— — — — —	— — — — 1.837(70)	— — — — —
— — — — 1.69 (F)	— (W) 1.785 — (M) 1.72 —	1.805(5) 1.778(60) — 1.704(5) 1.682(10)	— 1.78(30) 1.75(10) 1.715(40) 1.68(20)	— — 1.75(10) 1.715(38) 1.68(15)	— — — — —	— 1.76(20) — 1.72(80) —	— — — 1.72(40) —	— — — 1.71(16/12) —	— — — — 1.691(80)	— — — — —
1.66 (F) 1.63 (S) — 1.58 (F) 1.535(F)	— (M) 1.625 — (F) 1.60 (F) 1.56	1.659(80) — 1.616(70) 1.581(40) 1.539(60)	1.66(20) 1.63(100) 1.610(30) 1.590(10) 1.56(30)	— 1.63(100) — — 1.56(10)	— 1.633(100) — — 1.564(14)	— 1.64(30) — — —	— — 1.61(5) — —	— — 1.61(64/33) — —	— 1.634(10) 1.596(40) — —	— — — — —
1.50 (W) 1.485(S) —	— (M) 1.48 —	— 1.486(90) 1.464(10)	1.495(30) 1.485(80) 1.475(50)	1.495(30) — 1.475(50)	1.502(20) — —	— — 1.47(50)	— — —	— — 1.48(80/53)	— 1.484(70) —	1.523(60) — —

*The pattern of FeS given in Fig. 3(b) indicates a considerable quantity of free iron, some of whose lines are clearly visible, viz. those at 2.03A, 1.43A and 1.17A. Also, traces of the ordered phase are indicated by weak lines at 1.44A, 1.275A, 1.23A, 1.19A.

**These two faint lines in the pattern of the magnetic fraction are almost certainly the residual Kβ corresponding to the two very strong lines of the pattern at 3.00A and 2.70A.

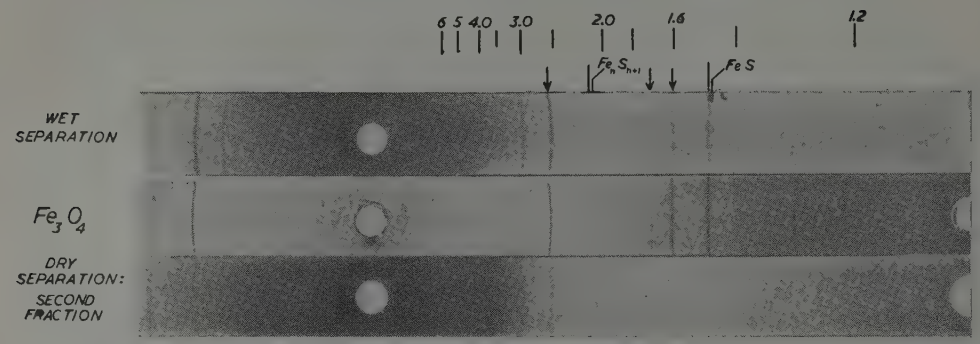

Fig. 4.—Comparison of the X-ray diffraction patterns of the magnetic fractions obtained by (i) wet and (ii) dry separation with the corresponding pattern of Fe_3O_4. Except for the two very weak lines marked Fe_nS_{n+1} and FeS, the close approximation of the wet separated sample to Fe_3O_4 is at once apparent.

patterns of the treated coal and of its first magnetic fraction are at 7.00A (I=70), 3.53A (I=60), 2.99 (I=80), 2.70 (I=60), 2.51 (I=100), 2.40A (I=80), 2.33A (I=70), 2.06A (I= 100), 1.63A (I=100) and 1.485A (I=80). The lines at 7.00A (I=70), 3.53A (I=60), and about half the intensity of that at 1.485A (I=80) would correspond as before to kaolinite. The outstanding lines, after subtracting the contribution (approximately one-half of the intensities given in the standard) due to all the lines of kaolinite, are shown in the 5th column of Table 4, and these are to be analyzed for the iron sulphides and oxides. The line at 2.51A is the strongest line of the Fe_3O_4 and α-Fe_2O_3 patterns, while strong lines at (1) 2.99A, (2) 2.70A and (3) 2.06A correspond respectively to (1) FeS, (2) FeS_2, FeS or α-Fe_2O_3, and (3) $Fe_nS_n+_1$. It follows that FeS and Fe_nS_{n+1} are both present. A comparison of the complete data of their standard patterns with the observed pattern was next made. The presence of a line of moderate intensity at 2.65A confirms the presence of $Fe_nS_n+_1$, while the lines at 2.33A and 2.095A confirm the existence of FeS.

The FeS contribution to the observed pattern is seen to be nearly 1/3rd of the intensities given for the standard pattern (Table 4), while the $Fe_nS_n+_1$ contribution is about 3/4th of the corresponding standard. From these relative intensities, it follows that the quantity of FeS is about 1/2 that of $Fe_nS_n+_1$. The presence of the very strong line at 1.63A can only be explained by the existence of (unreacted) FeS_2 in the treated coal, and this is confirmed by the strong to medium lines at 2.40A, 1.90A and 3.10A, which however overlap with weak lines of the patterns of Fe_3O_4, FeS and kaolinite, respectively. From the strengths of all the four lines, the quantity of FeS_2 can be estimated as nearly equal to that of $Fe_nS_n+_1$.

(ii) Oxides of Iron.—As mentioned earlier, the strong line observed at 2.51A corresponds to one of the strongest lines of both Fe_3O_4 and Fe_2O_3 (α- and γ-Fe_2O_3). For investigating the presence of α-Fe_2O_3 two of its medium to strong lines, viz. those at 1.84 (I=70) and 1.69 (I=80), which do not overlap with the strong lines of other phases, can be used as distinguishing criteria. It is found that, although a faint line at 1.68A does show on the pattern, the line at 1.84A is not observable. It can thus be estimated that the quantity of α-Fe_2O_3 would be less than 1/5th that of Fe_3O_4 (and / or γ-Fe_2O_3, whose pattern is practically indistinguishable from that of Fe_3O_4). Thus the major contribution to the strong line at 2.51A must come from Fe_3O_4 (and/or γ-Fe_2O_3) whose presence is further confirmed by a line of medium to weak intensity at 1.475A. From the intensities of the main lines of Fe_3O_4, many of which overlap with those of other phases, it follows that Fe_3O_4 would be present in somewhat less quantity than Fe_nS_{n+1}. FeO can be taken to be absent from the magnetic fraction because none of its strongest lines is to be found in the experimentally observed pattern.

5. Semi-Quantitative Estimates from X-ray Data

(i) Component Phases of the First Magnetic Fractions.—Having recognised the presence of Fe_3O_4 (and/or γ-Fe_2O_3) in appreciable quantities, it now follows this phase would partly contribute to the lines at 2.99A and 2.95A, which correspond to the main strong lines of FeS. Therefore, it appears that the earlier estimate of the quantity of FeS is too high, and after allowance is made for the contribution from Fe_3O_4 (and/or γ-Fe_2O_3) to this line, the revised estimate of the quantity of FeS may be put at about 1/3rd that of Fe_nS_{n+1}.

Thus it is seen that the magnetic fraction from the coal steam-treated at 400°C. contains four major iron-bearing phases, viz., Fe_nS_{n+1}, FeS_2, FeS and Fe_3O_4, the percentages of these being estimated as 5, 5, 2 and 4, respectively, from internal comparison of the intensities of their lines and using as an extenal standard a pattern of a mixture of 5% each of Fe_nS_{n+1}, FeS, Fe_3O_4 and FeS_2, with (80%) untreated coal. These figures lead to an estimate of 9% iron and 6% sulphur, which are in satisfactory agreement with the chemically estimated values of 8.8% and 7.2% Among the non-iron-bearing crystalline phases, kaolinite is the most important one, being present in quantities of the order of 30%. The rest is mostly carbon.

(ii) Comparison with the Previously Examined Magnetic Fraction.—The above results, which show that Fe_3O_4 constitutes only one quarter of the iron-bearing compounds in the magnetic fraction, contrast with the conclusion arrived at previously that Fe_3O_4 is the major crystalline phase in this fraction. Since the previously-studied material has been obtained by successive wet magnetic separations using a slow stream of water, a re-examination of this product was undertaken. A powder pattern of the old sample was taken with the 11.4 cm. diameter camera, using Cr K radiation. The pattern obtained is shown in Fig. 4 alongside of comparison patterns of Fe_3O_4 and the second (i.e. middle) magnetic fraction obtained in the present study.

Although, as noted in the preliminary study,[1] the pattern of the old magnetic fraction appears to be essentially that of Fe_3O_4, more careful examination shows that the three lines marked with arrows are diffuse, while in the other two prominent lines at 2.10A and 1.48A a distinct doubling can be detected, as indicated in Fig. 4 by the paired marks.* The stronger one of each doublet agrees with the corresponding line of Fe_3O_4, and the weaker ones are readily identified with the 2.06A line of Fe_nS_{n+1} and the 1.47A line of FeS, respectively. Confirmation of the existence of both these phases in addition to Fe_3O_4 is obtained from the diffuseness and strength of the 2.53A line, thus showing it to be a composite of the 2.53A line of Fe_3O_4 and the 2.54 A line of FeS. Weak lines can also be observed on the original pattern in positions corresponding to the other strong lines of FeS and Fe_nS_{n+1}. The strongest line of FeS_2 at 1.63A also appears to make a small contribution to the diffuse line marked with an arrow at this position. From the relative intensities of the several lines of the experimentally observed pattern, the ratios of Fe_3O_4, Fe_nS_{n+1}, FeS and FeS_2 can be estimated as 5:2:2:1. Comparison with the corresponding ratios 4:5:2:5 obtained above for the dry separated material show that the major part of the Fe_nS_{n+1} and the FeS_2 have most probably been carried away by the water in the wet separation process.

It may be concluded that the above combination of magnetic, chemical and X-ray analysis provides an adequate estimation of the important sulphides and oxides of iron that constitute the magnetic fraction of the steam-treated coal. At the same time, this magneto-chemical study throws some light on the progress of the reactions occurring in the conversion of the FeS_2 into Fe_3O_4 in the low temperature range of 300°C. to 400°C. It is hoped to follow up with similar investigations on some of the other Pakistani coals and their ashes, which are presently being studied in these laboratories.

Acknowledgements

The authors are grateful to Mr. Sadiq Ali Ahmad and Dr. G.H. von Hoessle of the Fuel Research Division for kindly supplying the samples of untreated and treated Makerwal coal and for providing some of the analytical data in Table 2.

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* These fine details were missed in the preliminary investigation reported earlier because the X-ray patterns were taken with a smaller camera and with copper radiation.

A PRELIMINARY NOTE ON THE SEED CONSTITUENTS OF CAESALPINIA BONDUCELLA

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(Received May 7, 1959)

The powdered kernel of bonducella nuts has been extracted successively with light petroleum (b.p. 40-60°C.), chloroform, rectified spirit and water at room temperature. The petroleum extract gives a fatty oil in ca. 20% yield, having an iodine value of 112; the oil deposits a jelly-like substance on long standing. From the chloroform extract, two new highly crystalline bitter substances α-caesalpin, m.p. 187° and β-caesalpin, m.p. 239-240° have been separated, while the alcoholic extracts yield sucrose in two crystalline forms, one cube and the other rectangular bar, besides some reducing sugars. A water soluble protein has also been obtained from the aqueous extract.

Introduction

Caesalpinia bonducella, or the commonly called fever-nut, grows wild all over the Indo-Pakistan sub-continent, especially in West Bengal, East Pakistan and South India, and is known locally by different names (Hindi, Karanjava; Bengali, nata or nata-karanja; Persian, khayae-i-iblis; Arabic, kitmakit). The common name for the seed is bonduc seed (bonduc in Arabic means a little ball or filbert). Every part of the plant is used medicinally, but in the present communication only the constituents of the seed have been discussed. The seeds are said to possess well-marked anti-periodic, antipyretic and tonic properties and were largely used by the local people as a substitute for quinine. They are considered by some to be useful for alleviating swellings and restraining hoemorrhage. Their application in leprosy and as an anthelmentic is also known.

Heckel and Schlagdenhauffen[1] isolated a non-alkaloidal bitter principle as a white amorphous powder from bonducella seeds, and assigned the name 'bonducin' to it. Bacon[2] said that bonducin was not a glucoside but a mixture of complex resinous bodies. In 1912, Bhaduri[3] reported to have isolated an alkaloid, 'natin' from the seeds. In an attempt to isolate the natin, Godbole et al.,[4] found that the bitter principle was not an alkaloid. He said that it was a sulphur-containing glucoside. Tummin Katty[5] obtained bonducin as an amorphous powder of indefinite melting point and concluded that the bitter principle was a complex non-alkaloidal and non-glucosidic mixture of resins; and Ghatak[6] stated that the bitter principle, bonducin, was a non-crystalline glucoside, $C_{20}H_{28}O_8$, m.p. 119-120°C., and it was neither an alkaloid nor a sulphur-containing glucoside. These varied opinions, as regards the nature of the active principle, are due to the fact that none of the workers was able to obtain the active principle in a pure and crystalline condition.

In view of the importance of bonducella nuts in the Ayurvedic system of medicine, and because of the very divergent views expressed by the earlier workers about the nature of its bitter constituent, a re-investigation of the seeds appeared to be desirable to find out the exact physiologically active component. This report consists of a complete preliminary account of the constituents of the fruit.

The fruit consists of a hard greenish grey shell, which has a white kernel. In immature fruit the colour of the kernel is brown and the constituents vary considerably. For the present investigation, only matured kernels have been used.

The kernel showed the presence of some fixed oil and was first treated to separate this oily constituent with a solvent. The amount of oil appears to be about one-fifth of its weight and is a very bitter substance. The bitterness can be attributed to the partial dissolution of the main bitter principle, mentioned below, in the oil. With the help of either hot rectified spirit or better in a charcoal column, this bitterness could be removed and the oil obtained as a clear colourless liquid mass, where the untreated oil was rather dark brown in colour. The oil has a fairly high iodine value and on standing for some time deposits a jelly-like product. The constituents of the oil are now under examination.

The defatted pulp was extracted with chloroform when a fairly neat sample of an extremely bitter substance came out. It was not one pure compound but appeared to be a mixture, m.p. 140-148°C., which was free from nitrogen and sulphur. Two bitter substances, one melting at 187°C., and the other at 239-240°C. have been separated from this mixture by fractional crystallisation from methanol. We have called the former α-caesalpin and the latter β-caesalpin; both of these are well-defined crystals. The quantities of the products are, however, rather small; these are

being accumulated and their detailed account will follow subsequently.

The debittered powdered kernel was next extracted in a percolator with rectified spirit, which removed a fairly large quantity of a sweet viscid mass. This on treatment with methanol, gave a well-defined crop of cubical crsytals, while another crop of crystals was obtained from the mother liquor as stout rectangular bars. Both the crops have been identified as sucrose, although literature records only one form of crystals (cubical) for the same. Besides this crystalline component, a syrup remained behind which reduced Fehling's solution and gave an osazone similar to glucosazone. So far it has resisted separation into crystalline components. However, besides the reducing sugar, it also contains a high proportion of non-reducing sugar, and other foreign materials which might have contributed greatly to the difficulty in the separation. Further work in this direction is in progress and the results will be communicated in due course.

The residue after alcohol extraction gave a considerable portion of it in water. When the aqueous solution was heated this portion coagulated into a white mass. It was found to be a water soluble protein while the rest of the solid material consisted of several other types of porteins which are now under examination and a report about it will follow later.

Experimental

Separation of Fixed Oil.—The sun-dried kernels of the matured seeds were ground to a fine powder, and 1000 g. of the same was percolated slowly for 5 days at room temperature with light petroleum (b.p. 40-60°C., 4000 ml.). Further percolation gave practically colourless oil-free extract. The combined percolates (3200 ml.) on removal of the solvent gave a brown oil (192 g., 19.2%) which was bitter to taste.

Purification of the Oil.—(i) The crude oil (200 g.) was vigorously shaken with hot rectified spirit (150 ml.) in a separating funnel. The mixture formed an emulsion which was set aside overnight whereupon two layers separated. The oily layer was treated twice in a similar manner, whereby it became completely bitterless but still contained an appreciable amount of colouring matter.

The alcoholic layers were combined and the solvent was removed under reduced pressure. The viscous residue after having been washed with light petroleum (40-60°C. 20 ml.) was dissolved in ether (10 ml.) and the solution kept in the refrigerator for 48 hours. The crystalline deposit (0.07 g.), m.p. 167°C., was collected and recrystallised from methanol, when an extremely bitter substance, m.p. 183°C., was obtained. Its mixed m.p. with the bitter principle of m.p. 187°C. was 186°C.

(ii) The petroleum extract (1200 ml.) from 500 g. of the kernel was diluted with the solvent (1000 ml.) and was then passed through a column (16 × 3 cm.) of animal charcoal (E. Merck), the rate of flow being regulated at 30 drops per minute. The solution emerged clear and colourless from the column . The adhering oil in the column was washed down with a little solvent. The combined solution and the washings were evaporated to a colourless and bitterless oil (113 g.). It showed saponification value of 190, and iodine value (Hanus) of 112.

The used charcoal was eluted with boiling rectified spirit (3 × 100 ml.) The eluate was freed from the solvent and the residual viscous mass was washed with a little light petroleum (b.p. 40-60°C.). Crystallisation of the semi-solid mass from ether (5 ml.) in the cold, followed by re-crystallisation from methanol, as before, gave the bitter (0.05 g.), having m.p. and mixed m.p. of 187°C.

Isolation of Bitter Principles.—The defatted kernel (originally 1000 g.) after removal of the last traces of petroleum ether by a water pump, was soaked with chloroform (1500 ml.) in a percolator and left at room temperature overnight. The extract was then run out of the percolator drop by drop during the whole day. The residual mass was again treated with fresh chloroform (900 c.c.) and the extraction operations were similarly carried out. This process of extraction was then repeated three times, after which only a bitterless and colourless percolate appeared to come out. The combined extracts (4200 ml.) were made free from the solvent by distillation under reduced pressure. The viscous residue was triturated with light petroleum (b.p. 40-60°C., 150 ml.) and the mixture was left in the cooler overnight. The solid residue (9.2 g.) was collected and washed with a little of the solvent. This was then dissolved in ether (40 ml.) and kept in a refrigerator for 48 hours whereupon a crystalline substance (0.7 g.; 0.07% on wt. of the kernel) deposited. It was collected on the filter, washed with a little ether and recystallised from benzene. It melted at 140-148°C.(not sharp) and gave negative test for nitrogen and sulphur. The substance was extremely bitter, readily soluble in methanol, ethanol, chloroform and acetic acid, but was very sparingly soluble in ether and petroleum ether and was moderately soluble in benzene. This was fractionally crystallised from

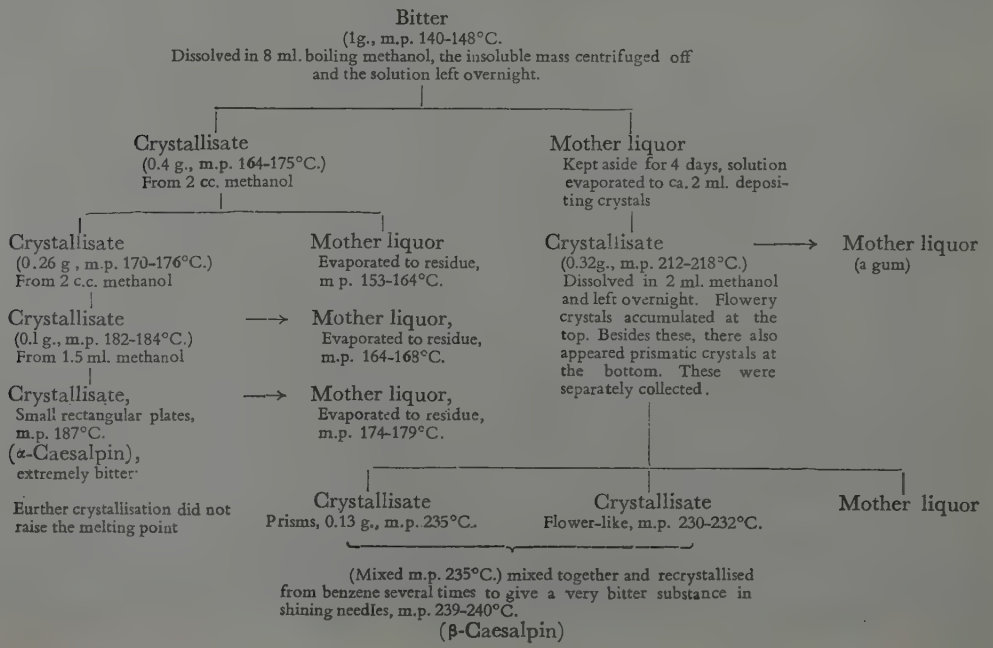
methanol as schematically shown in the chart. The above ethereal mother liquor was evaporated to an amorphous solid residue (7·3 g.) which could not be crystallised from different solvents or mixed solvents. It melted at 104-120°C. and was also extremely bitter to taste.

Isolation of Sugars.—The defatted and debittered residue of the kernel, after the above treatment was percolated with rectified spirit (7,600 ml.) at room temperature. The solvent from the percolates was removed by distillation first at ordinary pressure and then under vacuum. The viscous residue (73·5 g.) was then triturated once with absolute alcohol; a semi-solid mass was thus obtained. This was refluxed for several hours with methanol (300 ml.). The mass mostly went into solution leaving behind some crystalline solid. The mixture was allowed to cool and the crystalline solid (0.82 g.) was collected. This was dissolved in methanol (40 ml.) by boiling and the solution was filtered from the impurities. The clear solution on standing overnight deposited sucrose in small cubes, m.p. and mixed m.p. with a genuine sample of pure sucrose was 187°C., $[\alpha]_D^{32} = +66.69°$. Octa-acetate was prepared by the method of Linstead[7] when it melted at 88-89°C. Its mixed melting point with an authentic sample, prepared from pure sucrose, was not depressed. Thus the identity of sucrose is confirmed.

The methanolic extract (of the crude sugar) as obtained above was concentrated and allowed to stand, whereby some crystals in the shape of rectangular bars appeared. These were collected, and had m.p. 187°C. Its mixed m.p. with cane sugar was undepressed. They had $[\alpha]_D^{31} = 66.07°$ and gave an octa-acetate, m.p. 88-89°C.; mixed m.p. of this octa-acetate with sucrose octa-acetate was undepressed.

The methanolic mother liquor on further standing did not give any more crystalline solid. It was evaporated to a viscous mass which reduced Fehling's solution and also gave a small amount of an osazone, prepared by the method of Vogel,[8] melting at 205°C.; an admixture with an authentic sample of glucosazone did not depress the melting point. So far the sugar itself could not be crystallised and no crystalline acetyl derivative or *p*-nitrophenyl hydrazone has been obtained. It is therefore not possible at this stage to report its identity but more work on it is expected to give interesting results.

Estimation of Reducing and Non-reducing Sugars in the Non-Crystallisable Portion of the Alcohol Extract.—This estimation was carried out according to the procedure of H.K. Archbold and E. M. Widowson[9] involving oxidation with potassium hypoiodite before and after hydrolysis of the sample. It was


Bitter
(1g., m.p. 140-148°C.
Dissolved in 8 ml. boiling methanol, the insoluble mass centrifuged off and the solution left overnight.
Crystallisate
(0.4 g., m.p. 164-175°C.)
From 2 cc. methanol
Mother liquor
Kept aside for 4 days, solution evaporated to ca. 2 ml. depositing crystals
Crystallisate
(0.26 g , m.p. 170-176°C.)
From 2 c.c. methanol
Mother liquor
Evaporated to residue, m p. 153-164°C.
Crystallisate
(0.32g., m.p. 212-218°C.)
Dissolved in 2 ml. methanol and left overnight. Flowery crystals accumulated at the top. Besides these, there also appeared prismatic crystals at the bottom. These were separately collected.
Mother liquor
(a gum)
Crystallisate
(0.1 g., m.p. 182-184°C.)
From 1.5 ml. methanol
Mother liquor,
Evaporated to residue, m.p. 164-168°C.
Crystallisate,
Small rectangular plates, m.p. 187°C.
(α-Caesalpin),
extremely bitter
Mother liquor,
Evaporated to residue, m.p. 174-179°C.
Further crystallisation did not raise the melting point
Crystallisate
Prisms, 0.13 g., m.p. 235°C.
Crystallisate
Flower-like, m.p. 230-232°C.
Mother liquor
(Mixed m.p. 235°C.) mixed together and recrystallised from benzene several times to give a very bitter substance in shining needles, m.p. 239-240°C.
(β-Caesalpin)

found to contain 11.6% reducing sugar; calculated as glucose, and 46.17% of a non-reducing sugar, presumably sucrose.

Separation of Proteins.—The residue (25 g.) left after alcohol extraction was exhaustively extracted with water, 10% sodium chloride, 10% sodium hydroxide and 10% hydrochloric acid, successively. The water extract was heated on the steam bath for about an hour. The coagulates were collected and dried to give the water soluble protein, 19.7 g.; or 79% of the alcohol treated residue.

The sodium chloride extract, on being saturated with ammonium sulphate, yielded 1.75 g. or 7.02% of a protein on alcohol treated residue. The alkaline and the acidic extracts on neutralisation similarly gave 1.09 g. or 4.37% and 0.04 g. or 0.16% of proteins, respectively.

The residue, 1.86 g. or 7.42% on the alcohol treated residue, was very tough and appeared to be cellulosic in character. More detailed examination of the above four types of proteins is in progress.

Acknowledgement

We thank Dr. S. Siddiqui for his keen interest in the progress of this work.

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STUDIES ON THE THERMAL DECOMPOSITION OF ALKALINE EARTH SULPHATES

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(Received October 6, 1959)

Decomposition of alkaline earth sulphates as such and in the presence of various substances has been studied. It was observed as follows: calcium sulphate decomposed 0.53% at 900°C. and 3.15% at 1050°C. maintained for 4 hours; barium sulphate 3.93% at 1050°C. maintained for 4 hours; strontium sulphate 0.82% at 900°C. and 5.164% at 1050°C. maintained for 4 hours; and $MgSO_4.7H_2O$, 43.0% at 900°C. for 6 hours.

The maximum decomposition of alkaline earth sulphates in the presence of alumina in the ratio of 1:1 by weight at a tenperature of 900°C. maintained for 6 hours was noted to be $MgSO_2.7H_2O$, 88% and $CaSO_4.\frac{1}{2}H_2O$, 9.72%, the water of crystallisation being subtracted in each case. These results differ a good deal from those presented by some of the previous workers.

Decomposition of alkaline earth sulphates in the presence of chromium oxide, chromite ore, kaolin, bentonites, calcined graphite (free of carbon) and various other oxides were tried, and it was observed that some form of silica accelerated the rate of decomposition. Diatomaceous earth, a form of amorphous silica, was also tried, and the results confirmed the above findings. As diatomaceous earth is not available in our country, 'flue dust', a source of active silica and an easily available raw-material, was substituted successfully. This process therefore can usefully be employed for the production of sulphur dioxide and the residue used in cement industry on commercial scale.

Gypsum is found in large deposits all over the mountain ranges in West Pakistan. At high temperatures[2] and under suitable conditions gypsum can be decomposed as follows:—

$$2CaSO_4 \longrightarrow 2CaO + 2SO_2 + O_2$$

This reaction can be made use of in the decomposition of gypsum and other naturally occurring alkaline earth sulphates. But its industrial application would evidently depend on making the sulphates decompose at as low a temperature as possible and also as speedily and rapidly. Sulphur dioxide produced in the above reaction can be employed in various industrial processes.

The action of heat on gypsum has been studied by various workers from time to time during the last 30 years or so. Gervet[3] and his colleagues studied the effect of alumina on gypsum at 1400°C., Rana, Dube and Ratnam[4] studied the effect of temperature on gypsum and they obtained maximum production of sulphur dioxide upto 44.64%. Pailard et al.[5] studied the effect of kaolin on various sulphates for 6 hours in hydration, while Smirnov and Mehelov Patrosyan[6] studied the effect of heat on mixture of barium sulphate and alumina from 1200°C. to 1400°C., and the decomposition recorded by them was 40% and 93%, respectively, when the ratio was 1:1. Gruver[7] found the effect of heat on various sulphates on differential-thermal-analytical basis. Osmani and Datar[8] studied the effect of temperature on alkali metal sulphates in the presence of alumina and bauxite at 800°C. to 900°C. for 6 hours to get sulphur dioxide, and the same group of workers[9] also studied the effect of alumina and bauxite on alkaline earth sulphates. Sutten[10] and Manning and Maclennan[11] studied the effect of alumina, bauxite and silica etc. on gypsum at 900°C.

The present investigation was undertaken to study the action of heat on gypsum and other alkaline earth sulphates available in the country, with the object of decomposing them with various catalysts and other substances at as low a temperature as possible. For these studies, a thermogravimetric balance was designed so that more accurate observations could be made and a description of this instrument is also included.

Experimental

The materials studied were magnesium sulphate, calcium sulphate, strontium sulphate and barium sulphate. All the sulphates used in the actual experiments were laboratory grade chemicals. However, to facilitate extension of the work to the naturally occurring minerals, analyses of gypsum, celestite and barytes were carried out with the results given below. Some of the available catalyzing minerals, bentonites, koalin, chromite, graphite residue, diatomaceous earth and flue dust were also analysed for purity:—

1. Several samples of gypsum from Daudkhel were analysed and the average result is as follows: Free and combined water and loss on ignition, 25.366% (thermogravimetric balance); $CaSO_4$, 77.60%; SiO_2, 0.25%; Fe_2O_3, 0.07%; and Al_2O_3, 0.038%.

2. Only one sample of celestite from Daudkhel was analysed which gave the following results: Moisture and loss on ignition, 3.266% (thermogravimetric balance); $SrSO_4$, 80.30%; SiO_2, 11.0%; and CaO, 4.10%.

3. Average result given by the two samples of chromite ore is:—Moisture and loss on ignition, 4.290%; SiO_2, 11.0%; Cr_2O_3, 48.0%; FeO,

13.70%; and Fe_2O_3, 6.12%.

4. A sample of barytes was also analysed which gave the following results:—Moisture and loss on ignition, 4.13%; $BaSO_4$, 73.2%; SiO_2, 12.9%; and CaO, 2.2%.

5. Nine samples of kaolin were analysed which gave the following average results:—SiO_2, 45.17%; Al_2O_3, 38.25%; Fe_2O_3, 0.76%; and water content and loss on ignition, 14.9% (thermogravimetric balance).

6. Fourteen samples of bentonite gave the following average results: SiO_2, 53.96%; Al_2O_3, 10.36%; Fe_2O_3, 12.16%; Na_2O, 4.95%; and loss on ignition and water content, 15.9% (thermogravimetric balance).

7. Graphite residue was found to have the following composition: SiO_2, 80%; Al_2O_3, 11.2%; and Fe_2O_3, 5.5%.

8. Two samples of flue-dust from Daudkhel were analysed and were found to have the following composition: SiO_2, 50.5%; R_2O_3, 37.25%; and loss on ignition, 3.0%.

Decomposition of Pure Sulphates.—Sulphates with purity of E. Merck standard were used and the amount of water content was determined by two methods.

TABLE 1

	(a) Heating & desiccating	(b) Thermogravimetrically
$MgSO_4.7H_2O$	= 30.5 %	30.52%
$CaSO_4.2H_2O$	= 21.2 %	21.25%
$CaSO_4.\frac{1}{2}H_2O$	= 6.2 %	6.29%
$SrSO_4$	= 0.5 %	0.55%
$BaSO_4$	= 0.5 %	0.55%
$Al_2O_3.xH_2O$	= 3.06%	3.09%

The requisite proportion of the components were weighed accurately and ground for 1-2 hours in an agate mortar. The sample was then transferred to a silica boat and reweighed. The decrease in weight varied from 9 mg.—15 mg. for each sample. This correction was made in the calculations.

The followng heating arrangements were tried :—

(1) Heating in Gallenkamp Globar muffle furnace (0-1500°C.)

(2) Laboratory muffle furnace (0-1000°C.)

(3) Tube furnace (0-1100°C.). (Combustion nature).

Furnace (1) and (2) were later discarded due to some variations in temperature recorded with the help of the thermocouple-pyrometer arrangement. Furnace (3) of approximately 1" diameter was selected as it gave satisfactory results and so was used throughout the investigations. The furnace was equipped with a thermocouple pyrometer and a sunvic control. The samples were placed on the nickel half-round tube and introduced into the furnace and heated for a certain specified time at a fixed temperature. However the fluctuation was obvious which ranged ± 10°C. The samples were then taken out of the furnace, cooled in the desiccator and then weighed. The decrease in weight was recorded and the calculations were done on the basis of this decrease in weight, assuring it to be entirely due to decomposition. But as a cross-check the sulphate contents of the ignited samples were also determined gravimetrically. The results in both the cases showed close agreement. The decomposition of alkaline earth sulphates as such is given below:—

TABLE 2

Compound	Decomposition at 800°C. (For 6 hours)	900°C. (For 6 hours)	1000°C. (For 6 hours)	1050°C. (4 hrs.)
$MgSO_4.7H_2O$	6.802%	45.0%	100% in 45 minutes	—
$CaSO_4.2H_2O$	nil	0.53%	2.52%	3.15%
$SrSO_4$	nil	0.82%	3.03%	4.16%
$BaSO_4$	nil	nil	2.6 %	3.93%

There were some differences between the above results and those of other authors,[9] which could not be entirely due to experimental error. In order to be more systematic and accurate a thermogravimetric balance, designed and fabricated by the authors as described below, was used.

Thermogravimetric Balance.—To study the minute changes on heating in the weight of substances, a thermogravimetric balance was devised by Honda and his coworkers[13] in 1915. Later on, Guichurd and his colleagues,[14] Ghevenard and his associates in France, developed an automatic instrument of high precision and consequently a new technique of gravimetric analysis was introduced in the field of analytical chemistry.

As compared with Chavenard balance, a simple device was used by Burriel Mart; Barcia-Goyanes[15] at the University of Madrid. This consisted of a semimicrobalance in which one of the pans was replaced by a platinum crucible supported by a platinum wire. A thick layer of water separated the balance from the furnace. A sunvic control was used to regulate the temperature.

A similar set up with an analytical balance was used by the authors for the study of decomposition of alkaline earth sulphates. In the initial stages there were some difficulties which were mainly due to the following reasons:—

(1) The left pan of the balance was replaced by a paltinum wire and a stationary counterpoise. This decreased the swing which resulted in cotinuous contact of the agate point and the lower agate plate, consequently increasing the stability and decreasing the sensitivity. (2) The beam was slightly disturbed which resulted in erratic zero adjustment. (3) The platinum being a very good conductor of heat was affected even by the slightest variation in temperature, resulting in increase in the length of wire. (4) A thick layer of water was insufficient because it was heated after a few minutes which resulted in (a) disturbance in the level of the balance and (b) accumulation of water vapours in the balance.

In order to eliminate these defects, a modified thermogravimetric balance was devised by the authors for the determination of minute decreases in weight due to decomposition even at 1200°C.

Instead of replacing the pan by a platinum wire, contact of the platinum crucible was made through a hook attached at the base of the pan by a quartz fibre. This arrangement eliminated the abovementioned defects (1) and (2), viz., restored the sensitivity of the balance without increasing

Table 3.—Thermal Decomposition of $MgSO_4.7H_2O$* (1 g. in each case) in the Presence of the Following Substances with the Help of Thermogravimetric Balance at 900°C.

Reaction mixture	Ratio by weight	Moisture or water of hydration (mg.)	Decrease in mg. and % decomposition recorded after every hour												Total decomposition
			1		2		3		4		5		6		
			mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	
$MgSO_4.7H_2O$	1 : 0	302	54	11.7342	37	8.0401	36	7.8228	30	6.5190	26	5.6498	24	5.2152	44.9811%
$MgSO_4.7H_2O$ + Alumina	1 : 1	336	130	28.249	63	13.6899	62	13.4726	62	13.4726	59	12.8207	55	12.6034	94.3182%
$MgSO_4.7H_2O$**	1 : 0	302	9	1.9557	5.8	1.2603	4.6	1.0001	4.4	.9126	4.2	9.126	4	.869	6.8072%
$MgSO_4.7H_2O$ + Alumina**	1 : 1	336	62	13.4726	26	5.6498	20	4.346	20	4.346	19	4.1287	18	3.9114	35.8545%
$MgSO_4.7H_2O + Cr_2O_3$	10 : 1	302	80	17.384	54	11.7342	30	6.19	26	5.6498	24	5.215	22	4.7706	51.2726%
$MgSO_4.7H_2O + Cr_2O_3$	2 : 1	302	274	59.540	92	19.992	48	10.430	26.6	5.8902	16	3.4768	..	..	99.3290%
$MgSO_4.7H_2O$ + Kaolin	10 : 1	306	106	23.0338	68	14.776	42	9.0266	36	7.8228	30	6.5190	30	6.519	67.6972%
„ „ „	2 : 1	388	186	40.7178	115	24.9895	54	11.7342	34	7.3882	22	4.7806	20	4.3460	93.9563%
$MgSO_4.7H_2O$ + Graphite residue	10: 1	302	95	20.6180	42	8.1170	35	7.5980	32	6.947	20	4.346	16	3.472	51.1457%
„ „ „	2 : 1	302	314	68.2320	72	15.6456	23	4.998	12	2.6076	10	2.1730	10	2.1730	95.8100%
$MgSO_4.7H_2O$ + Chromite ore (ignited)	10 : 1	302	104	22.5992	68	14.7764	60	13.0380	48	10.4304	36	7.8228	32	6.9536	75.6804%
„ „ „	2 : 1	302	146	31.7258	106	23.0338	60	13.0380	39	8.4747	29	6.3017	24	6.2152	87.7882%

** 800°C.

* Decomposition of $MgSO_4.7H_2O$ (used) for a decrease of 1 mg. in weight = 0.2173%
„ „ $CaSO_4.\frac{1}{2}H_2O$ „ „ „ „ „ = 0.180 %
„ „ $CaSO_4.2H_2O$ „ „ „ „ „ = 0.215 %
„ „ $SrSO_4$ „ „ „ „ „ = 0.232 %
„ „ $BaSO_4$ „ „ „ „ „ = 0.262 %

stability, and eliminated the error due to blunting of the knife edge and also the danger of disturbance of beam resulting in erratic zoro. Use of quartz fibre eliminated defect (3), as the coefficient of expansion of quartz fibre is 0.256 as compared with 8.99 for the platinum[17]. Defect (4) was removed by a water circulating arrangement to maintain constant temperature throughout the experiment. In addition to the water circulating arrangement in the top of the chest, use of a water condenser was also made arround the quartz fibre to minimise the heat radiations. Whatever little heat escaped to the balance was cut off by the arrangement made below the pan.[18] The whole of the heating arrangement was enclosed in a chest made up of iron and asbestos sheets fitted on a table made for the purpose. The chest had the sliding doors which after setting the balance to work, were closed to prevent any influence of outside air. A sunvic control was also used to regulate the temperature, which was measured with a thermocouple and pyrometer arrangement. A magnifier was also put before the scale on the door of the balance, to detect even the slightest variation in weight easily. Silica gel was also placed in the balance to prevent the effect of moisture, if any. Sensitivity of the balance was 0.0001 g.

Thermal decomposition of $MgSO_4 \cdot 7H_2O$ in the presence of Al_2O_3 and other tried substances as found by thermogravimetric balance is given in Table 3.

Since the above results showed that the findings of the authors[9] were not reproducible with the arrangements with us, efforts were made to select a suitable catalyst or a promoter to bring about the decomposition in reasonable time and at easily attainable temperature.

Data of decomposition of alkaline earth sulphate in the presence of Al_2O_3, chromite ore, V_2O_5, Fe_2O_3, Cr_2O_3 etc. have been collected. The results of the above procedure though encouraging could not be taken as satisfactory. A major difficulty in the case of Cr_2O_3 was when added more then 50% in alkaline earth sulphate attacked the glaze, and so that experiments were conducted using chromite ore, taking it to be commercially in this field. The results though encouraging could not be taken as satisfactory, as high temperature up to 1050°C. was used for 34.48% decomposition of $CaSO_4$ in the ratio of 1:1 for 4 hours. The use of Al_2O_3 is also a possibility, but as it is not available in sufficient quantity, kaolin (a hydrated alumino-silicate) was tried instead. The results are given in Table 3.

TABLE 4.—THERMAL DECOMPOSITION OF ALKALINE EARTH SULPHATES (1 g. in each case) IN THE PRESENCE OF DIATOMACEOUS EARTH WITH THE HELP OF THERMOGRAVIMETRIC BALANCE AT 900°C.

Reaction mixture	Ratio by weight	Moisture or water of hydration (mg.)	Decrease in mg. and % decomposition recorded after every hour												Total decomposition
			1		2		3		4		5		6		
			mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	
$CaSO_4.\frac{1}{2}H_2O$ + Diatomaceous earth	1 : 0.32	64	35	6.30	24	4.32	17	3.06	10	1.08	10	1.8	7	1.26	17.540 %
,, ,, ,,	1 : 1	63.5	97	17.46	54	9.72	40	7.20	30	5.40	21	3.78	20	3.60	47.16 %
$SrSO_4$ + Diatomaceous earth	1 : 0.32	2.2	20	4.640	13	3.016	8	1.856	5	1.16	3	.696	2	.464	11.832 %
,, ,, ,,	1 : 1	2.5	30	6.960	15	3.480	13	3.016	8	1.856	3	.696	2	.464	16.472 %
$BaSO_4$ + Diatomaceous earth	1 : 0.32	2.5	13	3.406	8	2.096	5	1.310	3	.786	3	.786	1.2	.3144	8.6984%
,, ,, ,,	1 : 1	2.5	24	6.288	15	3.930	10	2.620	5	1.310	3	.786	2	.534	15.368 %
$MgSO_4.x\,H_2O$* + Diatomaceous earth	1 : 0.32	305	198	93.0254	80	17.3840	63	13.6899	29	6.2717	19	4.1287	10	2.1730	86.6677%
,, ,, ,,	1 : 1	305	232	99.9136	128	27.8144	63	13.6899	30	6.5190	10	2.1730	2	.4346	100.0385%
$MgSO_4.x\,H_2O$ + Flue dust	1:0.32	305	81	17.6013	72	15.6356	54	11.7342	18	3.9114	13	2.8249	10	2.1730	53.8804%
,, ,, ,,	1 : 1	305	166	36.0718	76	16.5148	67	14.5391	58	12.6034	42	9.0636	30	6.5190	95.3217%

*at 800°C.

Since the results are definitely better than those of Fe_2O_3, chromite ore etc., other alumino-silicates were tried for further improvements. Second in order was bentonite (a montmorilonite structure). Another substance available known as graphite residue of the composition given earlier was also tried for the decomposition with the results shown in the table.

It was found from the above table that there was a marked increase in the decomposition of alkaline earth sulphates in the case. Since there was a large amount of silica and alumina and small amount of Fe_2O_3 as compared with bentonites, it was necessary to find out which of the constituents contributes much in decomposition. For this purpose data were collected with mixture of Fe_2O_3 plus kaolin and bentonites in the ratio of (0.1:1) at 1050°C. for 4 hours respectively. From the tables it was inferred that Fe_2O_3 had little effect on the decomposition of alkaline earth sulphate. Data were also collected with mixture of alumina and kaolin and bentonite. It was further concluded that the presence of silica (amorphous) had much more tendency of decomposition of alkaline earth sulphate than alumina. So a form of amorphous silica, i.e., diatomaceous earth, was used for the decomposition.

Data have been obtained with the help of thermogravimetric balance for the decomposition of alkaline earth sulphates in the presence of diatomaceous earth at 900°C., and the results are given in Table 4.

Table 5.—Thermal Decomposition of Alkaline Earth Sulphates in the Presence of Flue Dust.

Reaction mixture	Ratio by weight	Temp. °C.	Time	Decomposition %
$CaSO_4$ + Flue dust	1 : 0.32	900	6 hrs.	14.4
,,	1 : 0.5	,,	,,	16.8
,,	1 : 1	,,	,,	20.3
,,	1 ; 0.32	1000	4 hrs	26.0
,,	1 : 0.5	,,	,,	32.7
,,	1 : 1	,,	,,	68.2
,,	1 : 0.32	1050	,,	38.8
,,	1 : 0.5	,,	,,	88.2
$SrSO_4$ + Flue dust	1 : 2.32	1000	,,	26.5
,,	1 : 0.5	,,	,,	31.5
$BaSO_4$ + Flue dust	1 : 0.32	,,	,,	24.03
,,	1 : 0.5	,,	,,	29.9
$MgSO_4.7H_2O$ + Flue dust	1 : 0.32	800	6 hrs.	See Table 1
,,	1 : 0.5	,,	,,	

As the diatomaceous earth is a rare substance it may not be possible to use it for decomposition on commercial basis. Another substance which is the flue-dust from the burning of indigenous coals of the composition given earlier was tried. The results obtained are given in the last two rows of Table 4 and in Table 5. The above results are most commendable for the utilization of alkaline earth sulphates on commercial basis at easily attainable temperature.

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DIFFERENTIAL SURVIVAL OF TWO RACES OF PHYTOPTHORA INFESTANS*

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(Received June 6, 1959)

The effect of forced air-drying for varying periods has been studied on race A and race BD of blight of potato fungus, *Phytopthora infestans* (Mont) de Bary. The very much greater resistance of race A is connected with its dominance.

Introduction

In nature, the late blight of potato fungus, *Phytopthora infestans* (Mont) de Bary spreads from one plant to the other by splashing of rain or they may be blown around by wind. If they fall on a potato leaf and moisture is present they quickly germinate to start new infection. In absence of moisture, sporangia soon dry and die. In nature, race A for *Phytopthora infestans* is more commonly found on susceptible varieties of potato as compared with other races, which are equally virulent on these varieties. The purpose of this experiment, in addition to demonstrating the general effect of drying, is to show the lower sensitivity of the prevalent race A to the absence of moisture as compared with a race less prevalent, viz., race BD, which might help to account for race A's dominance and survival.

Material and Methods

The material include 36 Cobbler potato plants in 4 inch pots, water suspensions of sporangia of race A and BD, hand sprayers, electric fans and incubators. Seed pieces were planted in flats and left to grow until 1 to 2 inches above ground, when 36 plants of uniform size were transferred to 4″ pots.

Potato plants were inoculated when approximately 14 inches high and having at least 4 well-formed leaves. The inoculum consisted of sporangia, which were harvested from cultures[1] grown on whole yellow peas at 20°C. for 2 weeks. The concentration of sporangia of each race was adjusted to give approximately equal concentration of sporangia, i.e., 30,000 and 50,000 per ml. for race A and BD, respectively.

One set of plants for each race was then sprayed with inoculums as uniformly as possible. All plants except the controls were dried after inoculation by placing them in front of fans for about 1½ to 2 minutes; then, after 5, 10, 20 and 30 minutes, sets of three plants were remoistened with a knapsack sprayer and placed in the incubators. The approximate temperature of the green house at the time of inoculation was about 74°F. and relative humidity between 66 and 68%. The plants were kept overnight in the incubators.

The lesions on the terminal and 2 lateral leaflets of each 4 leaves of each plant were counted 4 days after inoculation.

Results

Each treatment by either of the isolates were in triplicate. Results on the count of infection in all the three replicates in each set of treatment are given in Table 1.

Table 1.—Number of Lesions per Plant Inoculated with Races A and BD of *Phytopthora infestans*.

Race	Time of drying	Replicates i	ii	iii	Total
Race A	0 min.	179	265	185	629
	5 ,,	27	23	61	111
	10 ,,	27	23	40	90
	20 ,,	23	11	5	39
	30 ,,	1	1	12	14
Race B D	0 min.	218	160	215	593
	5 ,,	49	38	14	101
	10 ,,	5	10	5	20
	20 ,,	7	1	1	9
	30 ,,	0	0	0	0

The results in Table 1 show that the total number of lesions produced by race A on the controls are slightly higher than that produced by race BD. The effect of drying for 5 minutes is more or less equal on race A and BD of *Phytopthora infestans*. But after 10 minutes they differed: race BD consistently caused smaller number of lesions than race A, until after 30 minutes of drying race

* The experimental work was done in the Plant Pathological Laboratory of the University of Minnesota, Minneapolis, Minn., U.S.A.

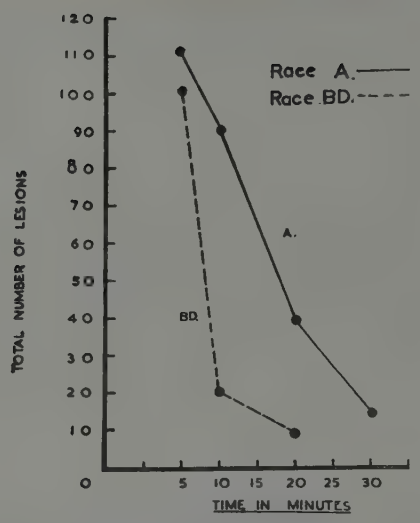

Fig. 1.—Effects of drying on infection potentiality of two races of *Phytopthora Infestans*.

BD produced no lesions while race A produced 14. Fig. 1 shows a graph of the results.

The results indicate that the sporangia of the race BD is more sensitive to drying than that of race A. Drying period over 5 minutes has a vital effect for race BD, when there is a sharp decline in the number of lesions from 101 to 20. On the other hand, the decline in the number of lesions produced by race A is gradual with increase in drying period. No lesions was found for race BD after more than 20 minutes drying.

Discussion and Conclusions

Race A of *Phytopthora infestans* can stand longer drying period than race BD as observed in the experiment and on the whole the number of lesions produced by race A for all drying periods are more than those of race BD, though initially concentration of race BD (number of sporangia per m/l) was slightly higher that of race A. After 20 minutes drying, no lesion was produced by race BD. All these indicate that, as race A can produce more infection than race BD and can withstand more drying period, it has a greater chance of survival and therefore spreads in the field under natural conditions.

Although other factors like overwintering, amount of sporulation, speed of producing germ tubes and so on may also play important roles for the prevalence of races in the field, yet ability to withstand dryness is most important of them all, and race A has this ability, which thus largely accounts for its prevalence under natural conditions.

A STUDY OF SOME INDIGENOUS PHARMACOPOEIAL HERBS

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(Received September 1, 1959; Revised December 4, 1959)

The description, occurrence, analytical data and important therapeutic properties of the following indigenous pharmacopoeial herbs have been given : *Podophyllum, Belladonna, Datura* and *Hyoscyamus.*

Introduction

There is no need to emphasize that the development and progress of a country depend largely upon the exploitation of its natural resources and raw materials. Among a multitude of these raw materials, nature has provided us valuable drugs but it will require extensive research and heavy expenditure of money before we are able completely to assess their medicinal value. Still there are a number of indigenous medicinal plants and herbs which have been fully investigated and their therapeutic value established. These herbs often grow in nature in the plains or hills of West Pakistan and the present communication gives important analytical data that has been obtained on several such plants.[3,4,5]

Podophyllum

One of the important indigenous drugs available in large quantities is *Podophyllum emodi*, which is locally known as 'Bankakri'. This member of the family *Berberidaceae* is a perennial glabrous herb. Its aerial stem is 6-12 inches high, having big peltate, long-stalked, alternate, curiously reflexed, umbrella-like and usually spotted leaves. The herb produces large, solitary, white and sub-sessile flowers in May, and bear bright orange-coloured fruits of the size of a small lemon, ripening in August or September.

The herb occurs in higher and interior temperate regions of the Himalayas at 6,000-8,000 ft. above sea level from Hazara to Murree Hills, Gallies, Kaghan, Kishanganga and Bhogarmung valleys descending to 6,000 ft. in Azad Kashmir. It is also fairly abundant in Malakand and Khyber, Kurram and Gilgit Agencies, Indus Kohistan and Swat State. It is plentiful on the northern moist forest-clad aspects of these areas especially where the sun shines less and is found scattered on the southern slopes of these mountains.

Experimental.—The estimation of resin from podophyllum was carried out by the following method which is an improvement over Jenkins' method.[2] It is easier to carry out and gives better results.

Weigh 10 g. of the drug in No. 80 powder and extract with alcohol (95%) in a Soxhlet apparatus for about 4 hours. Recover most of the alcohol till a little less than 50 ml. of the residue is obtained. Transfer it completely to a measuring cylinder and let it come to room temperature; make up the volume to 50 ml. Pipette out 10 ml. of chloroform and 10 ml. of water containing 0.1 ml. hydrochloric acid. Shake and let the chloroform layer separate. Draw the chloroform layer in a second separating funnel, and extract successively with 20 and 15 ml. of alcohol-chloroform mixture (1:2), adding each separated lower layer into the second separator. Shake the chloroform extracts with 10 ml. of the same acidulated water which was used before, allow to separate, take the chloroform layer in a tared crystallization dish, repeat the extraction successively with 20 ml. and 15 ml. of the above alcohol-chloroform mixture. Evaporate the total extractive on a water bath. Dry at 35 °C. and finally over sulphuric acid. Weigh, multiply this weight (2 g. of drug) with 50 in order to get the precentage of resin present in the drug.

The results obtained with *Podophyllum emodi* are given in Table 1.

A perusal of Table 1 shows that Pakistani podophyllum contains resin in commercially exploitable quantities and is considerably superior to the American podophyllum, in which the percentage of resin is only one third of that in the Pakistani variety. It is also superior to the Indian variety. The rhizome yields more resin than the rootlets. The best season for the collection of the drug is the end of August, when the fruit of the plant is about to ripe and contains a maximum quantity of resin.

Since the potency of the crude drug depends chiefly on the presence of a bitter, tonic and purgative principle,[5] known as podophyllotoxin, the percentage of this fraction is shown in

Table 1.—Resin in *Podophyllum emodi, Wall.*

Month of collection	Locality	Part analysed	Resin (%)	Resin (%) reported elsewhere
October, 1956	Shogran (Kaghan Valley), Southern aspect, 9,000 ft.	Rootlets Rhizomes Rhizome and rootlets	7.20 12.60 11.50	4.17-5.2 in *P. peltatum* (U.S.A.).
October, 1956	Naran (Kaghan Valley), Southern aspect, 8,000 ft.	Rootlets Rhizomes Rhizome and rootlets	8.10 12.00 10.90	9.55 in *P. emodi* (Kulu, East Punjab) India.
April, 1957	Naran (Kaghan Valley), Northern aspect, 7.500 ft.	Rootlets Rhizomes Rhizome and rootlets	9.98 11.10 10.99	11.12 in *P. emodi* (Chamba, East Punjab) India.
August, 1957	Sharan (Kaghan Valley)	Rhizome and rootlets	15.56	—
August, 1957	Sharan (Kaghan Valley)	Rhizome only	16.38	9.0 in *P. emodi* (Bashahar, East Punjab) India.

Table 2.—Podophyllotoxin in the Rhizome of Podophyllum.

Name of variety	Place of origin	Podophyllotoxin (%)
P. emodi	Sharan (Kaghan Valley)	3.90
P. emodi	,, ,,	3.19
P. emodi	Naran, (Kaghan Valley)	4.10
P. peltatum	U.S.A.[1]	0.77
P. emodi	Bashahr (India)[1]	3.50

Table 2, which reveals that indigenous podophyllum contains three times more podophyllotoxin than the American drug.

The estimation of the percentage of podophyllotoxin in the rhizome of podophyllum was done in this Laboratory by the methods of Elder and Schneiter.[5A] The resin (0.5 g.), prepared from podophyllum, was extracted with 15 ml. of chloroform and filtered. Ten ml. of the filtrate was poured in 80 ml. of petroleum ether in a tared flask. When the precipitate subsided, it was filtered through a tared Gooch crucible, and the precipitate and the flask were washed with 20 ml. of petroleum ether. The fractions of precipitate in the crucible and the flask were dried at 70°C. for one hour, weighed and the percentage calculated.

Solanaceous Drugs

Some important pharmacopoeial drugs like belladonna and hyoscyamus are obtained from several species belonging to various genera in the family, *Solanaceae*. These drugs are not only used for the preparation of galenicals and pharmaceuticals but are also employed for the manufacture of atropine which is one of the most important mydriatic alkaloids and finds wide application in ophthalmic surgery.[6] Sufficient quantities of these drugs can be collected from different localities in Pakistan. The present work was carried out on various samples of these drugs collected from different localities, to find out the possibilities of their commercial utilization in pharmaceutical industry.

Atropa acuminata Royle ex Lindley, or Indian belladonna, one of the most important of these herbs, is found wild in Indus Kohistan and parts of Azad Kashmir between 6,000-12,000 ft. We could not find this plant growing in Muzaffarabad as mentioned in some books.[1] The plant is known as Angur-e-shifa (Urdu), Yebrug (Bengali), and Mait brand (Kashmiri).

Some samples of belladonna were analysed according to the assay method of British Pharmacopoeia, 1953 and the results are given in Table 3.

Table 3.—Analysis of *Atropa acuminata* Royle ex Lindley.

Date of collection	Locality of collection	Part analysed	Total alkaloids % as hoscyamine	
			in local samples	in foreign species[7]
27-7-1956	Shahid Pani (Bhugarmung Valley)	Air-dried roots	0.67	0.47
15-8-1955	Darband (Indus Kohistan)	Air-dried leaves and flowering tops	0.60	0.45
Aug., 1957	Kamalband (Kaghan Valley)	Leaves only	0.30-0.66	0.45
—	Market sample	Roots only	0.54	0.47
25-7-1958	Experimental Farm, N.R. Labs., Peshawar	Fresh, air-dried leaves	0.54	0.45

Table 3 shows that *Atropa acuminata* contains a higher proportion of total alkaloids as compared with the foreign species. The specimens of roots analysed in this laboratory contain 0.54 to 0.67 percent of alkaloids as compared with 0.4 percent laid down in the British Pharmacopoeia, 1953 and the leaves contain 0.30 to 0.66 percent as compared with 0.3 percent. In view of the presence of suitable percentage of active principles, the drug available in nature can be utilised in industry.

Datura Stramonium L.

Datura stramonium, L. or thorn apple, which is locally known as Siah Dhatura (Urdu), Tor Datura (Pushto), and Kala Datura (Punjabi), is another important medicinal plant which is common in the foothills of Dir, Swat and Chitral States, Azad Kashmir, Murree Hills, Kaghan Valley, Gallies, Kurram Agency, Astore and upper Gurez Valley up to 9,000 ft. It is found scattered all over Gilgit Agency and western Tribal Territories. The plant is also reported to occur in some parts of East Pakistan where it is known as Sada Dhutura.

The results of the various samples analysed according to the assay method of Indian Pharmaceutical Codex, 1953 are given in Table 4.

It is evident from the above data that indigenous

Table 4.—Analysis of *Datura stramonium, L.*

Date of collection	Locality of collection	Part analysed	Total alkaloids % as hyoscyamine	
			in local samples	in foreign species[8]
14-9-1955	Baragali (Hazara)	Air-dried leaves	0.29	0.25
May, 1956	Abbottabad	Seeds	0.23	0.20
14-7-1956	Abbottabad	Seeds	0.29	0.20
Aug., 1957	Abbottabad	Air-dried leaves and flowering tops	0.23	0.25
11-6-1958	Experimental Farm, N.R. Labs., Peshawar	Fresh air-dried leaves	0.32	0.25

Datura stramonium can profitably be utilised for the manufacture of pharmaceuticals and other medicinal preparations because of its high alkaloidal content when compared with 0.2 and 0.25% in the seeds and leaves, respectively, as laid down by the different pharmacopoeias.

Another plant of datura genus is *Datura alba* Nees, called Sufaid Dhatura in Urdu and Spin Datura in Pustho. It is common all over the plains of the former N.W.F.P., lower hills of the former Punjab and Azad Kashmir up to 3,000 ft. It also occurs in the central parts of the Punjab and northern Sind and is found scattered in the waste places of West Pakistan and Karachi. Table 5 shows the alkaloidal contents of the drug worked out according to the assay method of I.P.C., 1953.

Table 5.—Analysis of *D. alba* Nees.

Date of collection	Locality of collection	Part analysed	Total alkaloids % as hyoscyamine	
			in local samples	in foreign species[9]
Sept. 1955	Peshawar	Roots	0.01	—
May, 1957	Peshawar	Air-dried leaves	0.25-0.3	0.22
Oct., 1957	Peshawar	Seeds	0.24	0.23
11-6-1958	Expt. Farm, N.R. Labs., Peshawar	Fresh air-dried leaves	0.14 hyoscine	0.32

Leaves of *Datura fastuosa*, L. cultivated at the Experimental Farm of these Laboratories, collected at different times of the year, were also analysed and the results are given in Table 6.

TABLE 6.—ANALYSIS OF *D. fastuosa Linn.*

Date of collection	Locality of collection	Part analysed	Total alkaloids % as hyoscyamine in local samples	Total alkaloids % as hyoscyamine in foreign species[8]
24-6-1953	Experimental Farm, North Regional Labs., Peshawar	Fresh air-dried leaves	0.109 Hyoscyamine	0.22
10-7-1958	,,	,,	0.112 Hyoscyamine	0.22
24-7-1958	,,	,,	0.120 Hyoscyamine	0.22
12-12-1958 (before sun-rise)	,,	Fresh leaves	0.260 Hyoscine	0.22

Tables 5 and 6 reveal that the total alkaloids of these two species are below the standard for the prepartion of galenicals, but, in view of the suitable environmental conditions in Pakistan for this herb, it is worth experimenting to improve its quality by cultivation.

Hyoscyamus Niger, L.

Hyoscyamus niger, L. (Henbane), or *Khurasani ajawain* as it is called in Urdu, is commonly found in Baluchistan, Gilgit Agency and Western Tribal Territories from 10,000-15,000 ft. *H. muticus*, L., another species of hyoscyamus, is common in Baluchistan, Khyber Agency and other tribal areas, and is found scattered in the former N.W.F.P. and Punjab. Some samples of this herb were analysed according to assay method of B.P. 1953 and the results are given in Table 7.

The above analysis of *Hyoscyamus niger* shows that it is up to the standard laid down by B.P. 1953. It is believed that the low alkaloidal content of the cultivated drug can be improved by adopting better methods of collection and cultivation.

Discussion

It will be observed that indigenous podophyllum can not only feed the local pharmaceutical industry but can also be exported as it can easily compete with the podophyllum of other countries in the world market, for the reason that the percentage of resin as well as the percentage of active principles in the resin is comparatively high. The drug should be collected at the end of August when the herb contains the maximum quantity of resin. Wild growing plants deserve the protection and control of the Government to preserve the natural sources. The cultivation of the herb should also be tried at suitable places like Abbottabad and other hilly tracts.

TABLE 7.—ANALYSIS OF *Hyoscyamus niger.*

Month of collection	Locality of collection	Part analysed	Total alkaloids % as hyoscyamine in local sample	Total alkaloids % as hyoscyamine in foreign species 7
July, 1956	Darband (Indus Kohistan)	Leaves and flowering buds	0.046	0.45
2-9-1956	Saiful Maluk (Kaghan Valley)	,, ,,	0.058	0.45
Aug., 1 57	Kaghan Valley	,, ,,	0.058	0.45
13-5-1957	Landi Kotal	Leaves and flowering tops of *H. muticus*	0.053	0.45
20 5-1958	Expt. Farm N.R. Labs., Peshawar	Leaves only	0.015	0.45
—	Market sample	Leaves and flowering buds	0.038	0.45

As regards solanaceous drugs, the Pakistani herbs, *Belladonna*, *Hysocyamus* and *Stramonium*, contain active principles up to the pharmacopoeial standard and can be utilised in the pharmaceutical industry. As the plants are not abundant in nature or are found scattered, it is advisable to start their cultivation at suitable places in the country. Under controlled conditions, it should be possible to improve the percentage of alkaloidal contents by improving the soil and manure etc., and to ensure uniform crops collected at the proper time.

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SCOPE FOR DEVELOPMENT OF FERMENTATION INDUSTRIES IN PAKISTAN

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(Received August 19, 1959)

A general survey of the field is given with emphasis on the applications of microbiological fermentation processes to the production of industrial chemicals, pharmaceuticals, foods, etc., in Pakistan.

An efficient utilization of surplus and waste agricultural materials is always an important consideration, particularly for an agricultural country like Pakistan. The majority of substrates used in the fermentation processes originate as products of agriculture. Carbohydrate materials such as molasses; wood wastes, like saw dust, wood chips and wheat, rice, oat and barley straws; linseed, mustard, sunflower and cottonseed cakes; corn cobs, vegetable and fruit wastes; textile and paper trade wastes, etc., can be utilized directly or indirectly as raw materials abundantly available for many fermentation processes. The fermentation processes include the production of organic solvents, ketones, organic acids, antibiotics, vitamins, enzyme preparations, proteins, fats and plant and animal growth factors as well as sewage disposal, curing of hides, retting of flax, silage making and those food products which depend on micro-organisms and enzyme systems for their character and flavour, e.g., cheese, cocoa and beverages.

Cane molasses, a by-product of the sugar industry is an important raw material for fermentation, but it is not being adequately used in the country at present. The present annual production is estimated at about 64,000 tons, and molasses is being sold at about Rs. 25/- per ton on the basis of the raw sugar present in it. With the establishment of more sugar factories a still bigger surplus of this versatile raw material will be available. On the other hand, in the West where molasses has been a boon to the fermentation industry, the present cost of molasses in the United States is 13 cents a gallon (approximately Rs. 100/- per ton) and is becoming far too high for economic use in many of the industrial fermentation processes. Since the Second World War, there has been a tremendous and apparently a permanent increase in the demand for molasses for animal feed.

More recently, finding no adequate industrial use for this material in the country, it was being planned to export cane-molasses to foreign counties to earn foreign exchange, but the limiting factor was high transportation costs from the factory sites to Karachi by rail. By initiating fermentation industries in the country, it would be possible to produce chemicals and other products of great economic value which are at present being imported.

During the latter half of the nineteenth century more fermentation industries were developed. The use of black mould in the tanning industry was shown to depend on the production of gallic acid from tannin by *Aspergillus niger*. Lactic acid was produced commercially in the United States as early as 1881 by modifying the existing processes for producing alcoholic beverages which were followed by acetone-butanol process. During the First World War, glycerol was produced in Germany and U.S.A. and soon after mycological production of citric acid on a commercial scale by the use of *Aspergillus niger* became possible.

Apart from the production of alcoholic beverages, industrial alcohol and glycerol, yeast is an excellent source for enzyme preparations such as invertase and the yellow enzymes, growth factors, bios I, bios II and vitamins especially those of the B-complex, including vitamin B_{12}. Ergosterol, the precursor of vitamin D, is also produced industrially from yeast. A more direct utilization of yeast for this purpose is the production of yeast itself for food. As nitrogen can be supplied in an inorganic form, this amounts to a most economical method of synthesizing protein.

Purified vitamin B_{12} is also being produced from *Streptomyces olivaceus:*, and it is also being recovered as a by-product in various fermentations as well as from activated sludge from sewage treatment farms.

The discovery of pencillin in 1929 and its utilization during the Second World War opened a vast field of new fermentation processes and techniques in the field of antibiotics. In a period of less than 10 years, starting with the first deep fermentation plant of pencillin in 1944, microbiological processes became firmly established in industry.

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A few classical examples of the utilization of bacteria are worth mentioning: coversion of ethanol to vinegar by the acetic acid bacteria, oxidative potentialities of *Acetobacter xylinum* in the oxidation of sorbitol to sorbose in the synthesis of ascorbic acid. Apart from these, several other bacterial fermentations have achieved commercial usages, one of these being the use of *Bacillus aceto-ethylicus*, the main end products being ethanol, acetone, formic acid, acetic acid, lactic acid and hydrogen. The products using *Clostridium acetobutylicum* are *n*-butanol, acetone, ethanol, carbon dioxide and hydrogen. By choosing different strains, it is possible to modify the ratios of solvents or to produce isopropanol instead or together with acetone. Blackstrap molasses, starchy mashes or vegetable wastes can be used as carbon sources in these fermentations. The solvents are removed by distillation, carbon dioxide is collected for conversion to 'dry ice' and hydrogen is used in the production of ammonia or methanol. The residue left after removal of the solvents is spray-dried and the resultant butyl stillage is a rich source of riboflavin and other vitamins.

The recovery of sulphur from gypsum by the action of sulphate reducing bacteria is an example of the utility of micro-organisms in making available material that is otherwise recovered only with great difficulty. The bacterial degradation of sewage to give inflammable gas (mainly methane) employing *Clostridium methanigene* is another process. Over 50% of the products are gaseous, consisting of a mixture of methane and carbon dioxide. Apart from this, these micro-organisms are a good source of vitamins of the B group, and may be regarded as an indirect method of vitamin production.

The earliest substance to be recognized as of microbiological origin is lactic acid which is still being produced entirely by fermentation; molasses may be used as a raw material. It finds use in the textile and tanning industries as a solvent and its new use would be mainly in the plastics field. At present, there is not a clear and direct connection between moulds and plastics, but it is noteworthy that itaconic acid is produced far more easily by moulds (*Aspergillus terreus*) than by purely chemical process. Interest in itaconic acid as raw material for the plastic industry becomes clear if its formula is compared with that of methacrylic acid, whose methyl ester is the monomer from which 'Perspex' is obtained by polymerisation.

Some other mould acids are also potentially valuable because of the large yields obtained, e.g. kojic acid, fumaric acid, acids of carolic acid series and propionic acid.

Vitamin C is also produced by *Aspergillus niger* but in quantities which are too small to compete with the synthetic process at present. The position is completely different with regard to the latest members of the B-group vitamins, e.g., B_{12} which is now mainly produced as an important by-product in the streptomycin industry and is unique in being produced solely by micro-organisms alongwith vitamins B_{12a}, B_{12b}, B_{12c}, and B_{12d}.

The formation of cortisone, used in the treatment of arthritis by selective oxidation of the steroid molecule, is only possible by micro-organisms. Another example of a different type of process is based on the power of certain micro-organisms to synthesize from sucrose the higher molecular weight polymers of glucose known as dextrans for use as a plasma volume extender.

As regards antibiotics, except for chloroamphenicol, all other antibiotics are produced mainly by fermentation processes commercially.

Microbiological processes which could be locally developed are categorised as under:—

(*i*) *Industrial Chemicals*.—In the field of industrial chemicals, ethanol, butanol and acetone are the most important. These are however meeting keen competition from synthetic processes from petroleum and natural gas as raw materials in Western countries. This industry can however thrive in Pakistan, where low-cost molasses will continue to be available for a long time to come. Similarly, scope still exists for production of glycerol by fermentation, as demand for this material for various industries is very great indeed, and the existing soap factories in the country will not be able to cope with the increased demand; at the same time, plants for its production from petroleum are intricate and expensive.

(*ii*) *Pharmaceuticals*.—Antibiotics, vitamins, organic acids, dextrans, together with microbiological transformation of steroids are important applications of fermentation processes; especially the production of antibiotics, which has become a leading fermentation industry. Several of these could be commercially produced locally.

(*iii*) *Enzymes*.—Work is also needed on preparation of various enzymes: diastase in the liquefying and saccharifying starch, in the brewing and fermentation industries, desizing of textiles, sizing of paper etc. Similarly, commercial proteinases from fungal and bacterial sources find varied uses in industry. Pectinases prepared from fungi find use in clarification of fruit juices and

preparation of fruit concentrates.

(*iv*) *Foods, Feeds and Beverages.*—Microbiological investigations are also essential as we develop our food industries, e.g. canning industry where spoilage and food poisoning problems are of importance. Microbiology of dairy products, use of antibiotics for preservation of meat and fish, yeast as a source of human and animal food, improvements of the methods of manufacturing powdered milk, butter and cheese.

(*v*) *Preservation of Materials.*—Fibres from natural sources, ropes, building materials from wood etc. are all subject to microbiol deterioration especially in the sub-tropical climate in the country. Investigations are needed in this field as well.

As has been mentioned above, some of the products are being produced by synthetic means and from petroleum and natural gas in the West. In Pakistan, as agricultural by-products and wastes are available in plenty, application of microbiological processes should find greater and greater use for production of materials useful for chemical, pharmaceutical and other allied industries.

SHORT COMMUNICATIONS

A NOTE ON PAKISTANI ARTEMISIA

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(Received August 28, 1959)

Pakistani artemisia has, on account of its high santonin-content, come into great prominence in the world drug market. Pakistan is the largest artemisia producing country in the world, and indigenous santonin-containing species grow very abundantly, covering extensive areas. There are two important sources of commercial supply—the Upper Kurram Valley and the north-western Kashmir in the Gilgit Agency, the available natural resources of each of which is capable of satisfying the entire world need of santonin. No other country can, in the present circumstances, compete with Pakistan in regard to the production and supply of good quality artemisia. In the case of Pakistan artemisias, the botanicals grow wild in nature and no special care or expense is involved in growing them.

The Kurram and the Kashmiri artemisias botanically belong to two distinct species. While the Kashmiri artemisia is *Artemisia brevifolia Wall*, the Kurram artemisia was discovered by the author in 1926, and the history of its discovery is given elsewhere.[1,2] The Kurram artemisia is named *Artemisia kurramensis Qazilbash*.[3] The two artemisias differ from *Artemisia maritima L* morphologically, ecologically and genetically.[4] They are also distinguishable from *Artemisia maritima* as regards geographical distribution, nutritional requirements and photoperiodic responses.

Artemisia brevifolia Wall., bearing flower heads in different stages of growth.

Flowering shoots of *Artemesia brevifolia Wall*, showing flower-heads at different stages of growth.

A fully developed plant of *Artemisia kurramensis Qazilbash* growing wild.

Artemisia maritima L typically is a halophyte, which flourishes in saline soil. *Artemisia kurramensis Qazilbash* is a xerophyte, which flourishes in alkaline loamy soil. Salinity in the soil adversely affects the healthy growth of *Artemisia kurramensis Qazilbash*. Plants of *Artemisia maritima L* raised from imported seeds did not do well in the alkaline loamy soil of Peshawar and ultimately died.

Artemisia maritima L in typical cases is a hexaploid. The number of chromosome is n=27 and 2n=54. *Artemisia kurramensis Qazilbash* is a diploid. The number of chromosome is n=9 and 2n=18. It is interesting to note in this connection that hexaploids derived artificially from *Artemisia kurramensis Qazilbash*, on treatment with acenaphthene, resembled *Artemisia maritima L* morphologically as well as genetically in all important respects. The artificially produced plants were sent to Kew Botanic Gardens for confirmation. They were identified as similar to *Artemisia maritima L*. The experimental procedure adopted in producing the hexaploids of *Artemisia kurramensis Qazilbash*, resembling *Artemisia maritima L*, is described elsewhere.[5] Nevertheless, it appears that Chaudri[6] has confused *Artemisia kurramensis Qazilbash* with *Artemisia maritima L*. His conclusion is arbitrary and is not based on any original judicious scientific studies. *Artemisia kurramensis Qazilbash* is now universally recognised as a well marked distinct species, and its systematic independance is fully established.[7,8,9] It has aroused great interest all the world over on account of its high santonin content.

In the Gilgit Agency, santonin-containng artemisia grows in the Rattu area, Gurez, Kamiri and its adjoining territories in the north-western Kashmir.[10] Artemisia covers vast areas of elevated plateau lands and mountain slopes. In the Kurram Agency also, artemisia grows wild in desert areas and is commonly met with around cultivated lands. It flourishes very well on the ridges of cultivated fields, where it can get some irrigation water, and without any additional cost, avail of the manurial treatment, necessary for the regular field crops. Lately the cultivation of artemisia has been largely extended to regular fields. Such a practice however

Artemisia brevifolia Wall., showing roots, and flowering shoots. Locality, Sissu-Khoksar. Altitude, 11,000 ft.

A fully developed plant of *Artemisia kurramensis Qazilbash* growing in a cultivated field.

A flowering shoot of *A. K. Qazilbash* bearing fully developed heads.

is undesirable in the present economy of the country, because it adversely effects the production of much-needed cereals and other cash crops, and unnecessarily promotes the overproduction of artemisia to the great disadvantage of the growers. In view of the demand for santonin and the availability of the drug from other geographical sources, it is imperative that the production of artemisia be restricted within reasonable limits. It is also important for the survival of the artemisia industry that measures should be adopted for growing only selected high-grade artemisia in suitable areas provided with the best cultural conditions. For this purpose, proper selection and breeding of the strains with best santonin-content are essential. Propagation of newly evolved physiological strains with highest santonin-content is most desirable for successful competition with scientifically advanced and industrially highly developed foreign countries. For commercial cultivation of artemisia, it is essential that due regard should be paid to the best environmental conditions, which directly or indirectly are conducive to the development of higher santonin content. Soil fertility, soil humidity, atmospheric humidity, light and temperature effects are important basic cultural factors.

Artimeria maritima L.

Artimeria maritima L.

For successful competition with other countries, it is also very important to adopt more economical methods of harvesting. The present practice of assorting the collections already freed from woody pieces of stems and branches into A, B and C grades is hardly necessary especially when material of all grades is to be utilized for manufacturing santonin. It is quite an expensive, time consuming and laborious affair. If the manufacture of santonin is taken up locally, the dried tops freed from thick woody pieces of shoots could be utilized directly for processing. This will undoubtedly cut down the expenses to a considerable extent, and much labour and time will be saved.

The extraction of crude santonin could conveniently and cheaply be taken up locally by the lime and water process. In this proceas, the roughly powdered raw material, mixed up with slaked lime is boiled with water and filtered, the filtrate is concentrated and then treated with dilute hydrochloric acid, the acidified solution being allowed to settle down and to form a resinous sediment. The sediment is collected, mixed up thoroughly with powdered charcoal and allowed to dry gradually by stirring with a rod, the dried powdery mass being then packed in suitable containers and sent out for the manufature of refined pure santonin.

If the processing of crude santonin were taken up locally, the transport charges would be cut down to a very large extent. At present, there is no control on the growing of artemisia in the Kuram valley. Different strains of artemisia with low and high santonin content are grown together. Production in approved areas could be much

improved qualitatively and quantitatively by judicious use of commercial fertilizers and the application of improved modern techniques and methods of breeding in propagating the best physiological strains of high quality artemisia. Natural fertilizers used occasionally by the local growers are not quite adequate for satisfying fully the basic needs of the soil. It is most important to keep the soil in a healthy productive condition, and adequate administration of ammonium sulphate has remarkably improved the yield qualitatively as well as quantitatively.

Japanese santonin has lately appeared in the drug market, the botanical source of the raw material being *Artemisia matritima L* growing on Hokaido islands. The seeds were brought in 1925 from Thuringen (Germany), where *Artemisia maritima L* is cultivated for the supply of raw material for the manufacture of santonin in Germany. *Artemisia maritima L* growing on Hokaido islands is much inferior to *Artemisia kurramensis Qazilbash* in respect of its santonin content, and attempts are now being made to grow *Artemisia kurramensis Qazilbash* in Japan. The work is progressing under controlled experimental conditions, and has so far reached only a limited commercial scale.

At present there are two factories in Pakistan engaged in the manufacture of santonin—one at Rawalpindi and the other at Quetta. The santonin factory at Rawalpindi has a refining capacity of producing 5,000 - 6,000 kilograms of santonin. Santonin is a valuable althelmintic for the elimination of intestinal round-worms. Several other drugs have been used as substitutes from time to time. Santonin has, however, stood the test of time and has upto this day maintained its sovereign position among the remedies for the elimination of intestinal round-worms. Santonin still enjoys universal popularity and is regarded as a cheap reliable drug of well established value and repute. The biggest competitor of Pakistan in the world drug market is the U.S.R.R. The seriousness of the situation should be fully realized and every effort should be made to preserve and promote the healthy growth and development of the artemisia and santonin industries in Pakistan, as a long range issue. For this purpose well organised research of a high technical standard is most urgently needed. The need of the time is to improve the quality of artemisia and to adopt more economical and uptodate methods of santonin manufacture.

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A SURVEY OF FRUITS IN THE NORTH-WESTERN REGIONS OF WEST PAKISTAN

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(Received July 27, 1959)

As varying soil and climatic conditions are met within the north-western regions of West Pakistan, fruits of different kinds are grown on a commercial scale in the area. Since their cultivation is increasing rapidly, the area would continue to hold an important position in respect of fruit production in the country. Commercial fruit growing depends upon a number of factors for its success. One of the essential conditions is the presence of a well-organised fruit and vegetable processing industry to take care of the surplus or otherwise unmarketable fruits, and thereby to ensure a fair return to the growers and a steady supply of products at a reasonable cost to the consumers.

A number of enquiries were received in the North Regional Laboratories from industrialists seeking advice on the location and capacity of fruit processing units which could be profitably established in the area; it was also desired to explore the possibilities of export of some products to European markets. In order to develop the fruit processing industry on sound lines, it is essential to know the quantities of various varieties of different kinds of fruits grown in the region, their quality and season of availability, but no reliable information was

available from any source in this respect. A detailed fruit survey was, therefore, undertaken to collect reliable data in respect of fruits grown in the region and thus be able to recommend establishment of new fruit processing factories in the area. The survey has now been completed in the Peshawar district. The data collected reveal the following:—

1. Fifty four varieties of different fruits are commercially grown in the district.

2. The total area under fruits is 13,743 acres and the total annual production is 28,50,960 maunds, distributed as below among the three tehsils.

Peshawar Tehsil: area, 5580 acres; production, 11,83,512 mds.

Charsadda Tehsil: area, 2583 acres; production, 6,58,818 mds.

Nowshera Tehsil: area, 5580 acres; production, 10,08,630 mds.

3. Plums, pears, oranges and peaches are the main fruits grown in the district. Other fruits like "loquat," grapes, pomegranate, figs, apple, quince, apricot, banana and dates are grown on a limited scale.

4. The annual production of plums, pears, oranges, peaches and other fruits is 12.23, 8.66, 4.11, 1.25 and 2.26 lakh maunds, respectively.

5. A number of varieties of each fruit are under cultivation. The leading varieties are:—

a) Plums: Fazl-e-Manani and Formusa.
b) Peaches: 6-A
c) Pears: Leconte and Batang.
d) Oranges: Red Blood and Local White.

6. Only 0.23 percent of the fruit product (in the district) is annually utilised for processing by the existing factories. The remaining quantities are sold fresh.

7. The present area under fruits works out to 3.5 percent of the total area sown in the year 1956-57.

8. Area under fruits has been increasing steadily. A further increase of about 7,000 acres is expected in a few years when an additional 2 lakh acres of land would be brought under irrigation as a result of various irrigation projects nearing completion.

The above survey has clearly indicated that steps should be taken to utilise larger quantities of fruits for processing. Products should be manufactured for use within the country as well as for export. This work would be taken up in the North Regional Laboratories in the near future when necessary equipment is received.

A NEW SPECIES OF ALTERNARIA ON CASSIA HOLOSERICEA FRESEN

A. M. M. JURAIR AND AZMATULLAH KHAN

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(Received October 10, 1959)

In January 1959, a specimen of diseased leaflets of *Cassia holosericea* was collected from Hub River Road near Karachi. The affected leaflets showed a few scattered spots on both the surfaces of the leaflets, irregularly round or elongated, light to dark brown and bounded by a slightly raised margin, rather deeper in colour. The spots, which are at first small, brownish and 0.5 mm. to 2 mm. in diameter, gradually enlarge, becoming ultimately as big as 0.8 to 1 cm. in diameter, irregularly zonate and covered by the dark fructification of the fungus belonging to the genus *Alternaria* (Fig. 1).

Fig. 1.—Leaflets of Cassia holosericea affected by *Alternaria cassiae*.

Conidiophores in tufts, straight or slightly curved, septate, simple or branched, measure 36.0-112.0 × 16.0-24.0 μ. Conidia obclavate, muriform, smooth, light to dark-brown, with the beak subhyaline, slightly constricted at the septum measuring 92.0-332.0 × 28.0-40 μ (Figs. 2 and 3).

A review of the literature showed that no species of Alternaria has so far been reported on *Cassia holosericea* from any part of the world. Therefore the species under study was suspected to be new. This was later confirmed by the Commonwealth Mycological Institute, Kew, Surrey, England. The name *Alternaria cassiae* is being proposed for this species which is described below:

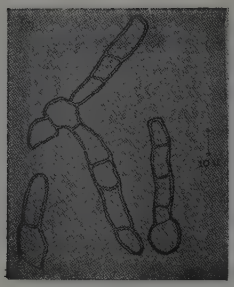

Fig. 2.—Conidiophores.

Fig. 3.—Conidia.

Alternaria cassiae spec. nov.—Maculae rarae, dispersae in utramque paginam foliolorum, irregulariter circulares vel elongatae, pallide vel fusce brunneae, circumdatae margine paulum elevato sat fusco colore diametientes primum 0.5-2 mm. gradatim evadentes latiores usque ad 0.8-1 cm. diam. zonis irregualribus ornatae atque opertae fructificationibus fuscis. Conidiophori glomerati, recti vel tenuiter curvati, simplices vel ramosi, septati, magnit 36.0-112.0 × 16.0-24.0 μ. Conidia obclavata muriformia, laevia, pallide vel fusce brunnea, rostro subhyalino ornata constricta ad septa, magnitud 92.0-332.0 × 28.0-40 μ.

Habitat: On the leaves of *Cassia holosericea* Fresen collected on 11.1.59.

Locality: Hub River Road near Karachi. Collected by A.M.M. Jurair.

Type specimen: Deposited in the University Herbarium, Karachi (No. 564), Co-type deposited in Herbarium Commonwealth Mycological Institute, Kew, Surrey, England under No. 75872 for record.

The authors are highly indebted to Dr. S.Z. Hasanain, Head of the Department of Botany, University of Karachi, for providing all facilities and encouragement; to Father H. Santapau, S. J., Professor of Botany, St. Xavier's College, Bombay, for the Latin description of the fungus reproduced above; and to the Director, Commonwealth Mycological Institute, Kew, Surrey, England, for confirming the identification.

A NOTE ON THE OIL CONTENT OF SOME KARACHI SARDINES

SHAIKH ABDUL AZIZ

Central Fisheries Department, Government of Pakistan, Karachi

Introduction

Information on the composition of fish is of basic importance to the solution of a large number of problems in the field of fish technology, and is essential for taking a decision on adopting any particular method of preservation, or using it as food or preparing industrial products from the whole fish or its wastes.

Since the compsotition, especially the oil content of fish shows wide variation depending

TABLE 1.—PROXIMATE COMPOSITION OF SARDINE, GREY MULLET, DRUMS AND SEA BREAMM.

Name of the species	Local name	Popular English name	Proximate composition							
			Protein (N×6.25)		Ether extract or Fat		Total ash		Moisture	
			Range	Average	Range	Average	Range	Average	Range	Average
Sardinella sindensis	Tarli	Sardine	18.5-19.0	18.7	1.0-3.0	2.0	1.3-2.2	1.7	76.5-78.9	78.0
Sardinella longiceps	Dome	Sardine	18.5-22.0	19.5	1.2-4.9	3.0	2.0-2.5	2.1	71.6-76.8	75.5
Otolithes ruber	Mushka	Drums or Croakers	16.5-18.2	17.2	0.9-1.3	1.0	1.2-2.5	1.3	75.5-78.5	77.5
Mugil speigleri	Boi	Grey Mullet	20.5-22.5	21.0	1.0-2.3	1.8	1.5-2.5	1.7	74.3-77.1	75.4
Chrysophrys sp.	Dandia	Sea Bream	19.0-20.8	20.0	1.3-2.8	1.9	1.5-2.5	1.9	70.9-74.5	74.0

upon the environmental conditions, seasons and other factors, the proximate composition of five species of locally available small fish along with some preliminary findings on the seasonal variation in the oil content of sardine are presented in this paper.

Methods and Material

Proximate analyses were carried out on each sample using standard techniques of the Association of Official Agricultural Chemists.[1] The fish used for these analyses were obtained from the local market and all the determinations were made on fresh unpreserved fish.

Results and Disccusions

The aproximate composition (protein, oil, ash, and moisture) of five varieties of small fish of an average length of six inches is shown in Table I, and some data on the seasonal variation in the oil content of sardine are given in Fig. I.

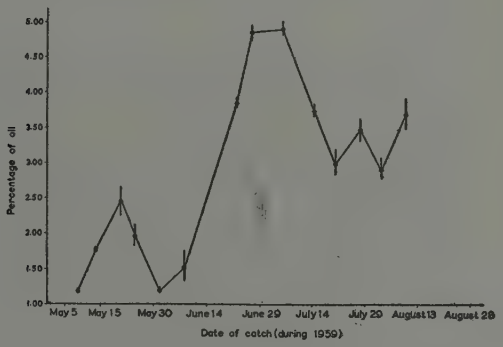

Fig. 1.—Seasonal variationa in the oil contedt of Sardine (*Sardinella longiceps*.

It will be seen from Table I that though the range of protein content in all the species of fish is fairly small, the oil content varies appreciably. The variation is the largest in the case of oil sardine (*Sardinella longiceps*) when it varies from 1.2 to 4.9. It will also be seen that the average oil content of sardine is much higher than the other three species analysed. This species (No. 2) also contains a high percentage of ash. Various experiments by other workers[2,3,4] have also shown that all constituents of fish change proportionately from time to time, but the constituent that undergoes the greatest changes is the oil or fat. This is due to the fact that many species of fish when feeding store oil as a reserve, to draw upon it later in the breeding season or migration. Such fish generally migrate over vast distances, and during these migrations they derive their energy from the fat reserve. This is the cause of seasonal variations in fat content. A summary of the observed seasonal variation in the oil content of sardine (*Sardinella logiceps*) is shown in Fig. 1. The maximum oil content as determined during the months of May, June and July was 4.9%. According to the classification of oily and non-oily fish, *Sardinella longiceps*, as used in this work, would fall under a non-oily class.

Acknowledgements.—The author is grateful to the Director of Scientific and Industrial Research, Karachi for giving facilities for work in the Central Laboratories, and to the Director, Central Fisheries Department, Government of Pakistan, for his kind permission and helping him in identifying the fishes used in this experiment, and also to Agha Ghulam Hussain, Deputy Director, Central Fisheries Department, for his keen interest and contained encouragement thoughout these investigations.

References

1. Association of Official Agricultrual Chemists, *Offcial Methods of Anaalysis* (Association Official Agricultural Chemists, P.O. Box 540, Benjamin Franklin Station, Washington, 4, D.C., 1955), eighthedition.
2. Karrick, Clegg and Stansby, Composition of Fresh Water Fish,Com. Fisheries Rev, **18**, No. 2 (1956).
3. M.E. Stansby and J.N. Lemon, Studies on the Handling of Fresh Mackerel (Scomber Scombrus.) U.S.F.W.S., Res. Rept, NO.1 1941.
4. Fatema Shaikhmahmud and N.G. Nagar, J. Sci. Ind. Research (India), **16**, (1957).

NOTES & NEWS

Deliberations of the Scientific Commission of Pakistan

The first meeting of the Scientific Commission was inaugurated by the President, Field Marshal Mohammad Ayub Khan, on the 4th August, 1959. In his inaugural address the President observed: "In his efforts to master nature, man through his own thinking and ingenuity, has undergone three important revolutions. The first was the Agricultural Revolution, thousands of years ago, when man learnt to sow, cultivate and harvest the food crops. The second was the Industrial Revolution of the 19th century which gave man the means of harnessing steam-power for locomotion, electric generation and production of goods by machines. The third is the Scientific Revolution of the present century which has not only led to a reappraisal of all values of life, material and spiritual, but has brought man nearer his goal of the mastery of nature." With regard to the Scientific Revolution, he remarked, "We are just beginning to feel its impact and the sooner we realize the benefits of its all-pervading influences the better. As a result of this revolution, science has emerged almost as a faith on which societies are being built today." The President further said that his Government was determined to lift the status of scientists in society and to offer the right careers to young men devoted to the pursuit of scientific knowledge and research.

Mr. Abul Kasem Khan, the Chairman of the Commission, referred in his address to some of the problems connected with the growth and development of scientific research in Pakistan and the application of its results in the various sections of national effort relating to the welfare of its people. He emphasized the necessity of wise planning and its effective and vigorous implementation in organising scientific research in the country.

Immediately after the inauguration, the Commission in its first few meetings drafted a comprehensive questionnaire, which was circulated among individual scientists, educational institutions and heads of various laboratories and scientific institutes in the country. The Commission also extended invitations to eminent scientists of the United States, the United Kingdom, Canada, Australia and Japan to come to Pakistan and participate as advisers in the deliberations of the Commission. The following scientists accepted the invitations and their visits to Pakistan were arranged under the Colombo Plan:—

1. Prof. A. V. Hill, F. R. S., Research Associate, University College, London; formerly Research Professor of the Royal Society; (United Kingdom).

2. Dr. A. L. Jordan; Professor of Chemistry, Royal Academy of Arts; formerly Director, Paint Research Station, Teddington; (United Kingdom).

3. Dr. Sakuji Komagata; formerly Chairman, Japan Atomic Energy Institute; (Japan).

4. Dr. F. T. Rosser; Vice-President, Administration, National Research Council, Ottawa; (Canada).

5. Mr. F. G. Nicholls; Research Secretary, Commonwealth Scientific and Industrial Research Organization, Melbourne; (Australia).

The Commission along with the advisers from overseas visited educational institutions, research institutes and laboratories in both wings of the country, and simultaneously collected evidence by interviewing experts and other people connected with scientific activities and scientific education at different levels. The Commission completed this tour of the country by the 9th December, 1959, and are presently outlining a draft report.

New Journal

To meet the need for rapid publication of communications in organic chemistry in preliminary form, a new international journal, *Tetrahedron Letters*, has been introduced from March 1959. Sir Robert Robinson and R. B. Woodward are Co-Chairmen of the Honorary Editorial Advisory Board, and the journal is published by Pergamon Press Ltd., London and New York.

This journal contains preliminary communications announcing experimental or theoretical results of organic chemical interest which are considered to be novel and significant. They may also be of a critical or speculative nature, and in that event are assessed for their positive value and

degree of originality before acceptance for publication. The aim of the journal is to publish accepted communications within seven to thirty days of acceptance.

While *Tetrahedron Letters* is published every two to four weeks, *Tetrahedron* is to continue to appear monthly for permanent contributions to organic chemistry.

Building Research in India

The overall impression produced by a study of the annual report for 1958-59 of the Central Building Research Institute, Roorkee, India, is one of purposeful activity on a very practical level.

Slightly more than half of this 42-page report is devoted to very brief accounts of research programmes in progress or completed in the Institute. Most of the fifty or so items of research thus reported are on problems of direct concern to potential users of research results, i.e. to builders, manufacturers, structural engineers and architects. Some of these research items have already gone into practical application, notably a roof construction using doubly-curved shell units, which gave considerable savings in cost and construction time in a one-crore housing project. In an introduction to the report, however, the Director of the Institute observes that while satisfactory progress has been made in setting up five of the planned divisions —Building Materials, Design and Performance of Structures, Soil Engineering and Foundations, Efficiency of Buildings, and Information—the formation of the sixth division, Building Practice and Productivity, is being delayed on account of the difficulty of recruiting suitable engineering and architectural staff; and that without this division the Institute is handicapped in its efforts to help industry to use the results of research. This situation is, of course, not unfamiliar in countries in which many industries are not yet sufficiently research-minded to provide their own research "interpreters" by recruiting to their staff, at good salary levels, scientists who have the necessary interest and ability in production and practice. The introduction to the report mentions that special steps are being taken to solve this vital problem, but does not indicate the nature of these steps.

The second half of the report summarises the year's activities in institutional matters, lists the papers published or read at meetings during the year, and includes an account of development of the Institute's library, a list of staff, etc.

At only one or two points is the balance of the report as a brief informative review of the year's developments upset by the inclusion of research detail that would fit more appropriately into a specialised scientific paper. The report is illustrated by well-chosen photographs.

(A.L.G.)

Forthcoming International Conferences

1st International Congress on Metallic Corrosion; April 10-15, 1961; London, England.

13th International Symposium on Crop Protection; May 7, 1961; Ghent, Belgium.

General Assembly of the International Organization for Standardization. (Members only); June 8-18, 1961; Finland.

European Convention of Chemical Engineering 1961. 30th Meeting of the European Federation for Chemical Engineering; June 9-17, 1961; Frankfurt-am-Main, Germany.

21st Conference of the International Union of Pure and Applied Chemistry; August 2-5, 1961; Montreal, Canada.

18th International Congress of Pure and Applied Chemistry; August 6-12, 1961; Montreal, Canada.

5th International Congress of Biochemistry; August 10-16, 1961; Moscow, U.S.S.R.

10th Pacific Science Congress; August 21 to September 2, 1961; Honolulu, Hawai.

6th International Conference on Co-ordination Chemistry; Ausgust 27 to September 1, 1961; Detroit, Michigan, U. S. A.

The Fourth International Congress on Coal Preparation. (Undertaken jointly by the National Coal Board and the Coal Preparation Plant Association); May 28 to June 1, 1961; Harrogate, Yorks, England.

7th International Conference on Co-ordination Chemistry; June 25-29, 1961; Stockholm, Sweden.

International Congress of Microbiology; August 19-25, 1961; Montreal, Canada.

PATENTS ABSTRACTS

Abstracts of patent specifications of Pakistani inventors notified as accepted in part IV of the Gazette of Pakistan during October 10, 1958 to December 25, 1959

108239 *An improved clay plaster and bricks.* A. H. Chotani and M. Aslam.—This Journal, **2**, 77 (1959).

108246 *A process for the production of cottonseed oil based coating compositions.*—S. Siddiqui and M. Aslam. (Australian patent 218,208, April 8, 1957; British patent 813,955, April 23, 1957; Canadian patent 592,615, April 13, 1957; Indian patent 60100, April 3, 1957.—This Journal, **1**, 250 (1958).

108270 *An improved wood preservative.* S. Siddiqui, M. Aslam and A. Mehdi. (Indian patent 60367, April 30, 1957).—This Journal, **1**, 250 (1958).

108274 *A process for the modification of tannin extracts for the manufacture of writing inks.* S. Siddiqui, G. Hahn and R. A. Siddiqui. (Indian patent 60781, June 14, 1957).—This Journal, **2**, 77 (1959).

108318 *An air entraining agent for cellular concrete.* S. Siddiqui, M. Aslam and S. J. Quadir. (Indian patent 60368, April 30, 1957).—This Journal, **2**, 77 (1959).

108319 *An emulsion type mimeograph ink and a new stabiliser therefor.* S. Siddiqui and M. Aslam. (Australian patent 221,488, June 19, 1957; British patent 834,311, May 20, 1957; Canadian patent 578,503, August 9, 1957; Indian patent 60366, April 30, 1957).—This Journal, **2**, 77 (1959).

108322 *A process for the simultaneous production of refined shark liver oil and vitamin A concentrates alongwith the recovery of other therapeutically active by-products.*S. Siddiqui, S. Mahdihassan, S. M. Ali and S. A. Haq. (Indian patent 60782, June 14, 1957).—This Journal, **2**, 77 (1959).

108339 *A process for making photographic colour pictures from a black-and-white photograph.* A.M. Saqi.- The process comprises in first dyeing the black-and-white photograph in a textile dye which will impart colour to all the gelatine and the white to grey parts of the picture, then destructing the dye from the opaque or exposed parts of the picture in proportion of the gradation of the silver density in a solution of acid thionrea or acid sulphocyanide, bleaching the silver image in a solution of ferrocyanide and bromide, redeveloping in a colour-developer derivative of para-phenylenediamine with a coupler of desired colour to obtain a second colour on the opaque parts of the picture in proportion of the gradation of the silver density, and after dyeing the image in two colours once again bleaching the silver and removing it entirely in hypo solution, thereby obtaining a two colour picture from a single black-and-white photographic image. A three colour picture can be obtained by dyeing one image in one colour and another image in two colours.

108448 *A solar engine.* C. A. Aziz.—The solar engine comprises an air-tight double walled solar vaporizer fitted with a pressure guage to show the pressure of vapours, a small chamber for collecting the vapours, a glass tube indicator to indicate the height of working substance in solar vaporizer, vapour outlet pipe fitted in the small chamber to let out the vapours, two inlet pipes fitted in the space between the walls, one for pumping the condensed working substance in the vaporizer during the day time, the other for letting in the working substance at night, an outlet pipe connected with a long pipe fitted with two taps, fitted at the bottom of vaporizer to lead out the working substance, the vaporizer having inlet at the top for filling in the working substance, its outer wall being coated with blackened sand and the inner side of the inner walls being insulated against loss of heat.

108473 *A pickless lock.* S. M. Y. Shah.—The lock has a cylindrical recess to house a bolt and nut mechanism which is formed at one end of the shackle of the lock, a bolt having a buffer at one end of the bolt of shackle which buts against the flangenut from falling apart, the flangenut being rotated by coacting key which operates the movement of the shackle to pick and lock it.

108527 *A choola using firewood or cowdung cakes.* The late M. Ishaq and C. K. Ilahi. (Indian patent 65343, September 23, 1958).—This Journal **2**, 78 (1959).

108539 *A process for the isolation of alkaloids and alkaloidal complexes from the Rauwolfia species of plants.* S. Siddiqui. (Australian patent 222,436, July 8, 1957; Belgian patent 566,712, April 11, 1958;

British patent 816,655, July 3, 1957; Canadian patent 609,833, July 5, 1957; Indian patent 60994, July 3, 1957; Japanese patent 30611, December 11, 1957).—This Journal **2**, 77 (1959).

108540 *A pharmaceutical composition for the treatment of hypertension.* S. Siddiqui, R. Deininger and M. Alauddin. (Australian patent 218,332, July 9, 1957; Belgian patent 565,811, March 18, 1958; British patent 816,654, July 3, 1957; Indian patent 60995, July 3, 1957).—This Journal, **2**, 78 (1959).

109040 *A liquid composition for destroying insects, their eggs and larvae.* A. A. Kazmi.—The composition is prepared from kerosene oil base about 9 parts, encalyphis oil about 1 part, pyrethrum extract (20%) about 2 parts, and turpentine about 3 parts.

109150 *A whirling wheel.* Maqsud Ali.—The wheel consists of a combination of a wheel of any material and a frame of two parallel mild steel wires or strips along which the wheel rotates, the wheel having a permanent magnet fitted in its axial bore with two mild steel bits with conical ends adhering to both the ends of the magnet protruding out of the bore and sewing as the axle of the wheel so that when the wheel is placed on the frame each end of the axle bears on each strip or wire of the frame and rotates along it when given a slight jerk or declivity.

109167 *An antimalarial medicine and a prophylactic against malaria.* A. A. Kazmi.—A chemical compound having the above-mentioned therapeutic properties is prepared from Swertia chiretta and alum.

109344 *A process for the production of a composition for water-proof sheets.* S. Siddiqui, M. Aslam and A. Mehdi. (Indian patent 65207, September 11, 1958).—A mixture of molten bitumen and a polymerised drying or semi-drying oil is heated till the mixture becomes homogeneous, and a filler is then added.

109346 *A process and an apparatus for the production of water-proof sheets.* A. H. Chotani and S. M. Ishaq Ali. (Indian patent 65868, November 18, 1958).—Jute or any other fabric is steeped in a molten waterproofing composition, and the resulting sheet, after being pressed under heated rollers, is dried in air and then quenched under a spray of water.

109353 *A calender for calculating conception days.* M. Ashraf.—The calender comprises four rotatable concentric discs bearing suitable marks on circumference so as to indicate conception days when the discs are adjusted properly.

109362 *An angethi using charcoal or soft coke.* The late M. Ishaq and C. K. Ilahi. (Indian patent 65344, September 23, 1958).—The angethi comprises a grate fixed in a casing having perforations, a closeable air inlet and a removable outer casing.

109400 *Biri wrappers (a substitute for biri leaves).* Jamiluddin Ltd.—Tobacco stems and roots and paddy straw are chopped into small pieces and are kept in water for about 12 to 18 hours. The resulting material is boiled in water for 6 to 8 hours, and sodium chloride and sodium bicarbonate are added while the material is being boiled. The material is then washed and pressed into paper form.

109415 *An Oil burner for heating and lighting purposes.* A. M. E. Manji.—The burner comprising concentric, perforated drums forming a chamber for mixing gasified oil with air is characterized by a ring fuel tank which surrounds the burner and the drums in their whole or partial height, leaving between the inner wall of the tank and outer drum a cylindrical space through which air can enter and reach the flame in the combustion chamber.

BOOK NOTICES

Biochemistry of Wood. Edited by K. Kratzl and G. Billek. 300 pp. Pergamon Press, London, New York, Paris, Los Angeles, 1959. Price 70s. ($11).

Biochemistry of Wood is Volume II of the edited proceedings of the Fourth International Congress of Biochemistry, held in Vienna, published in 15 volumes for the International Union of Biochemistry.

Wood is one of the most important organic raw materials. It has three main components—cellulose, hemicellulose and lignin. The botanical morphological method of examination was recently enlarged through the localisation of certain chemical structure elements with the aid of physical chemical methods of measurement.

The papers in this book review these advances, and give particular attention to the biogenesis of

cellulose. It is also noted that the biochemical approach has been particularly successful in the case of lignin, not only from the point of view of biogenetic conception but by enlarging the classical organic chemical structure theory. The last section of the book is devoted to biological formation of humus material.

These papers were intended to stimulate ideas on the biogenetics of wood's three main constituents. Their publication will undoubtedly carry this aim even further.

Partial Table of Contents: Conifer Chemistry and Taxonomy of Conifers—H. Erdtman; Terpene Formation in Pine—R.G. Stanley; On the Enzymic Polysaccharide Synthesis—G. A. Greathouse; Biosynthesis of Hemicelluloses—A. C. Neish; The Process of Lignification in Woody Plants—A. B. Wardrop and D.E. Bland; Studies on the Biosynthesis of Lignin—T. Higuchi; Lignification—F. F. Nord and W. J. Schubert; Zur Phylogenese des Lignins—S. M. Manskaja; Biochemie des Holzes : Ein Bericht ùber Symposium II—K. Kratzl.

The Biochemistry of Development. Professor Jean Brachet. 296 pp. Pergamon Press, London, New York, Paris, Los Angeles, 1959. Price 65S. ($10).

The Biochemistry of Development introduces the reader to the recent advances made in the field. This discussion of the main problems which now lie ahead will be useful for many advanced students and teachers, especially since there has been no text book in chemical embryology for almost ten years.

The Biochemistry of Development follows the development from the formation of the gametes to the differentiation of specialized organs such as lens or muscle. The accent is always placed on the more biochemical aspects of embryonic development; however, the recent morphological findings, made with the electron microscope, have not been left out, and the book presents one integrated morphological and biochemical picture of development.

Partial Table of Contents: Chapter I. Gametogenesis. General Metabolism; The Role of Follicle Cells in Yolk Formation; Chapter II. Fertilization. Morphological Changes at Fertilization (a) The Acrosome Reaction (b) Morphology of Cortical Changes During the Fertilization of Sea Urchin Eggs. Chapter III. Cleavage. Morphology and Cytochemistry of the Dividing Egg (a) Chromosomes (b) The Achromatic Apparatus (Spindle and Asters) (c) Ultra-structural Changes During Cleavage (d) Short Description of Frequent Mitotic Abnormalities During Egg Cleavage. Chapter IV. Chemical Embryology of Invertebrate Eggs. Ctenophores; Worms; Molluscs (a) Limnaea (b) Mytilus eggs (c) Ylanassa and Dentalium Eggs. Chapter V. Chemical Embryology of Vertebrate Eggs. Energy Production in Vatebrate Eggs (a) Effect of Anaerobiosis and Metabolic Inhibitors on Development (b) Oxygen Consumption (c) Local Differences in Oxygen Consumption (d) Qualitative Metabolic Changes During Development: Respiratory Quotient and Carbohydrate Metabolism (e) Experiments on the Incorporation of $^{14}CO_2$ (f) ATP Production and Utilization (g) Distribution of Mitrochondria. Chapter VI. Biochemical Interactions Between the Nucleus and the Cytoplasm during Morphogenesis. A Brief Summary of Theories on Nucleocytoplasmic Interactions. Chapter VII. The Biochemistry of Differentiation. Archencephalic and Spinocaudal Inductions.

The Chemistry and Technology of Edible Oils and Fats. Edited by John Devine and P.N. Williams. 225 pp. Pergamon Press, London, New York, Paris, Los Angeles, 1960. Price 35S. ($6.50).

This volume contains the important proceedings of the 1959 Conference sponsored by Unilever. It includes both the papers read and the authoritative discussions which followed them.

This is a subject of increasing importance in certain fields of medical and industrial research. This volume will throw new light on many aspects of the subject.

Contents: List of participants. Physical and chemical properties of the constituents of edible oils and fats—G.C. Hampson and B. J. F. Hudson; Fatty foods and the pattern of their consumption in the United Kingdom—R. J. Brech; Processing of oils and fats for edible purposes—P. N. Williams; The analysis of oils and fats—A. Crossley: The determination of linoleic acid with special reference to the isotopic dilution technique—G. J. van Beers; Gas/liquid chromatography, and its appllication to the analysis of atheromatous plaques—J. G. Keppler; Modern spectroscopic and X-ray techniques applied to oils and fats—D. Chapman.

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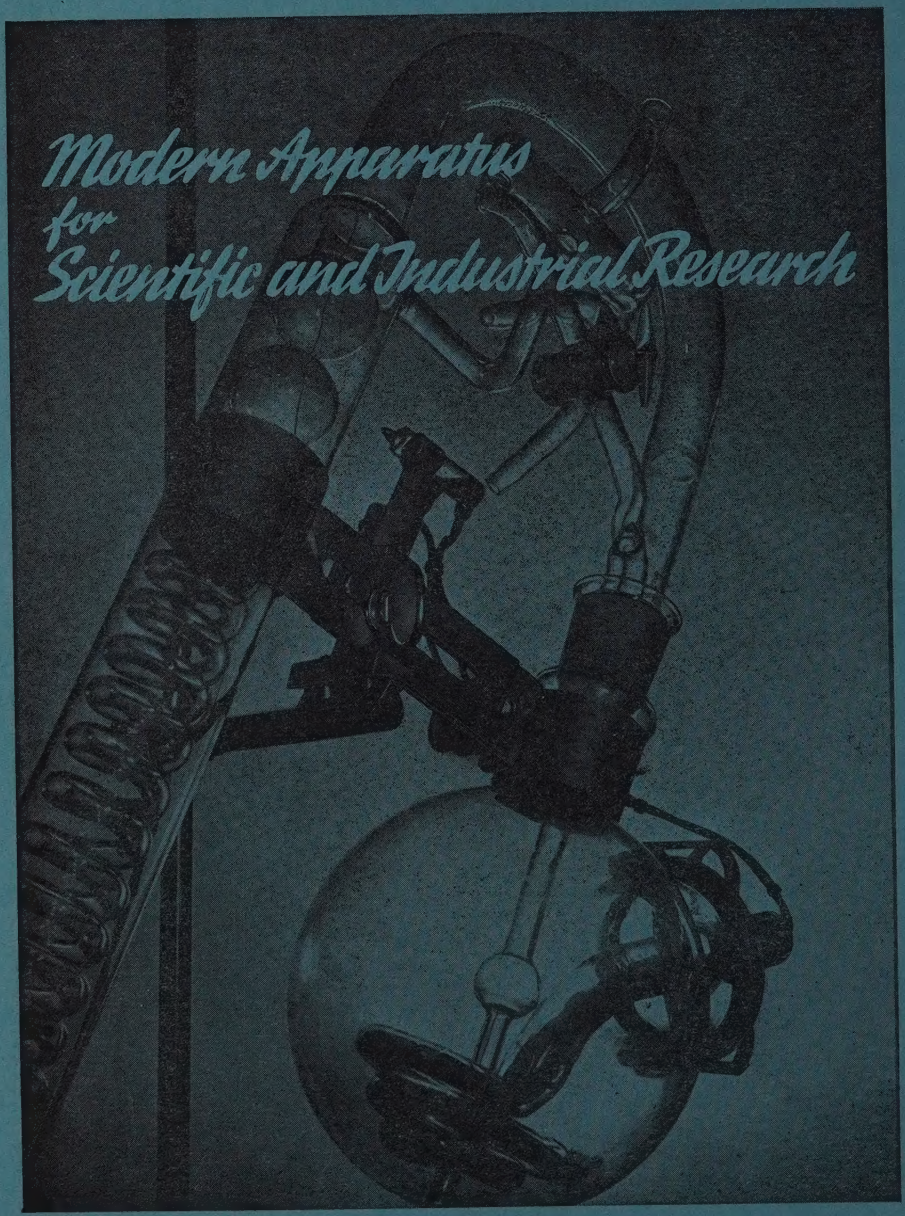

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Printed at Nazeer Printing Works, Water Course Road, Karachi